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The American Phytopathological Society

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PHYTOPATHOLOGY

VOLUME 25 NUMBER 2

FEBRUARY, 1935

VARIABILITY OF MONOSPORIC CULTURES OF COCCOMYCES HIEMALIS[1]

R. O. MAGIE[2]

(Accepted for publication June 19, 1934)

INTRODUCTION

The literature dealing with variation in fungi is rather extensive. Nearly all of the investigations in this field have been reported within the past 40 years. The conception of the fixity of a species, dominant until the latter part of the last century, probably accounts in large part for the fact that fungus variability was not studied earlier.

Schroeter (38) in 1879, working with certain rusts on Carex, was one of the first to observe that there were physiological forms or, as he termed them, "sister species." The first important demonstration of this phenomenon was made in 1894 by Eriksson (14) in his cross-inoculation experiments with *Puccinia graminis* Pers. on several species of grain. Ward (47) in 1902 found this pathogenic variation in *Puccinia dispersa* (Eriks.) Syd. on bromes. Between 1902 and 1903, Neger (32) in Germany, Marchal (28) in France, and Salmon (37) in England, reported pathogenically distinct forms for some of the powdery mildews on grasses and grains. The phenomenon of heterothallism in the mucors was demonstrated in 1904 by Blakeslee (2).

The work of these investigators opened up the importance and possibilities of the study of fungus variability and ushered in a series of investigations that have firmly established the fact of variation within a fungus species for fungi representing several families in both the ascomycetes and basidiomycetes. It is difficult to name a parasitic fungus in which variability has been sought but not found. It is now coming to be the common expectation to find much variation between different isolations of any fungus species.

The work on fungus variation has been directed mainly toward patho-

[1] Approved for publication by the Director of the Wisconsin Agricultural Experiment Station.

[2] The writer is particularly grateful to Dr. G. W. Keitt and to Dr. B. M. Duggar for their helpful advice and criticisms during the course of the investigation and the preparation of the manuscript.

genic fungi showing physiological specialization on their host plants. The knowledge gained by the study of physiological specialization has proved very important from several standpoints. As pointed out by Stakman (41), this knowledge is essential to the development of varieties of plants resistant to pathogenic fungi; to the understanding of the epidemiology of a disease; and to the establishment of judicious and adequate plant quarantines.

Work on fungal variation has also been done with saprophytic fungi and certain pathogenic ascomycetes and imperfects that exhibit their variability only in culture. The study of fungal variation in culture has proved useful in stimulating interest and study in the genetics of fungi; in providing a means for the study of sex in fungi; in mycological taxonomy; and sometimes in giving the first clue to possible host specialization.

There is some disagreement as to the mechanism by which heritable variations arise. Various workers have attributed this phenomenon to 1. Mutation, Stakman and co-workers (42), Brown (4), and Stevens (44); 2. Hybridization, Stakman and co-workers (42); 3. Segregation from a heterogeneous parent or heterokaryosis, Hansen and Smith (17) and Brierley (3), 4. "Dauermodification," Jollos (22) and Caldis and Coons (6); and 5. Dissociation from cyclic phases, Leonian (27). The difference in the theories as to the nature and cause of variability may be partly due to the fact that their sponsors have worked with different types of organisms and have used different procedures in studying their variation.

The purpose of this investigation was the study of variations exhibited by monosporic cultures of *Coccomyces hiemalis* Higgins, the fungus causing the leaf spot disease of sweet and sour cherries. An investigation of the epidemiology and control of this disease has been in progress for several years at Wisconsin. A study of the variability of this fungus seemed desirable to further the knowledge of the epidemiology and control of the disease, to initiate a study of the physiology of the pathogen, and to contribute to the foundation for further inquiries into the physiology of its parasitism.

Variability in the monosporic cultures was sought in their morphology, physiological reactions, and pathogenicity on several species of Prunus.

PHYSIOLOGICAL STUDIES

Methods and Materials

Monosporic cultures of *Coccomyces hiemalis* were isolated by the method described by Keitt (23) from sour cherry material obtained between 1930 and 1932 from Oregon, New York, New Jersey, Pennsylvania, Michigan, and several localities in Wisconsin. The cultures were carried on a medium

consisting of a water extract of 100 grams of cracked Lima beans, 10 grams of agar, and one liter of distilled water.

The cultures were numbered in the order in which they were isolated: Isolate Nos. 1–66, 86–90, and 103–113 from Wisconsin; 67–82 and 97–101 New York; 83–85 Michigan; X1–X59 Oregon; 91 Pennsylvania; and 92 New Jersey.[3] All isolates are from single ascospores, except 1 and 36–66, which are from single condia. No difference was observed between monoconidial isolates as a class and monoascosporic isolates.

Stock cultures on Lima-bean agar were stored at 9° C.[4] Transfers were made every 2–6 weeks. When carried on Lima-bean agar with sufficient transferring, most of the isolates slowly lost their vigor. This lack of vigor was manifested in a slower growth rate, fewer spores produced, and perhaps, finally the loss of sporulation. To obviate this difficulty those cultures used more intensively in these studies were passed through the host at least twice during the course of the investigation. These monosporic reisolations differed from the cultures used for the inoculations only in an increased rate of growth, increased sporulation, and sometimes a darker shade of color.

In most of the studies on physiology a standard medium modified from Leonian (26) was used both as a liquid and a solid substrate. The medium contained:

KH_2PO_4	0.4 per cent	Dextrose	2 per cent
Peptone	0.4 per cent	Distilled water	
$MgSO_4$	0.05 per cent	pH 5.5	

In order to obtain good growth, 1 per cent of liquid malt extract was added to this in most of the work. 1.7 per cent agar was added to make the solid substrate. Cultures in the liquid medium were grown in 250 cc. Erlenmeyer flasks containing 50 cc. of the medium. Each flask was seeded by adding 2 drops of a heavy spore suspension obtained by scraping off the spores of 2-week-old Lima-bean-agar slant cultures into 5 cc. of sterile distilled water. Dry weights of the fungal growths were obtained, with a variation of 1.5 mg., by filtering the liquid culture through 7 cm. Schleicher and Schuell filter papers, No. 589, washing with water, and drying over night at 95°.

Petri dishes containing 25 cc. of agar were seeded by transferring small masses of spores from Lima-bean-agar cultures by means of a needle, giving a seeded area of 1–2 mm. in diameter. All cultures were incubated at 20° unless otherwise stated. All cultural comparisons were made of material incubated in dark control chambers.

[3] The writer wishes to thank Dr. M. P. Backus for several of these cultures.

[4] All temperatures noted in this paper are in degrees Centigrade.

Because of the very slow growth of the fungus, both liquid and solid media cultures were usually incubated for 6 weeks before final data were taken.

Colony Variations

Each isolate differed from other isolates in certain characteristics that varied greatly with the medium used, the temperature, and the age of the colony. When grown on the standard agar with malt extract for 4 weeks at 20°, the isolates could be classed according to their colony color as follows: 1, 11, 47, 52, 87, 91, and X25 as flesh pink; X2, X12, and 21 as reddish pink; 20 and 58 as buff; 5, 6, 17, 24, 101, X14, and X27 as light brown; 27, 29, 43, 45, 49, 66, 69, 76, 77, 83, 92, X53 as dark brown; 8, 30, and 90 as orange; 3 as black; and 7, 13, 105, 110, and X46 as white. These latter were white from the time of isolation from single ascospores. Some of the other isolates produced white sectors soon after being isolated, or when they were allowed to become stale in culture from lack of sufficient transferring. White sectors and white isolates produced no spores and never reverted to a sporulating or colored condition. Temporarily white colonies were produced by some of the isolates when grown on potato-dextrose agar.

When cultures, grown for 2 weeks at 20° on Lima-bean agar or on the standard agar, with or without malt extract, were placed at 4°–8° for 3 weeks, the following isolates produced a dark-colored or black colony and blackened the medium: 6, 17, 24, X14, 27, 29, 43, 45, 49, 66, 69, 76, 77, 83, 92, 101, X27, and X53. These are all classed as light or dark brown in color. The other isolates, excepting No. 3, were never observed to produce a dark-colored pigment.

Isolate 3 differed markedly from all other isolates in producing a black mycelium and darkening the medium during the first few days of growth at 20° C. in the standard liquid and solid media and on Lima-bean agar. The colonies of some of the isolates, listed as light brown and dark brown, developed black centers after 4–6 weeks when grown on the standard and Lima-bean agars at 20°. This blackening of the centers of the colonies was hastened at lower temperatures.

The isolates were observed to differ also in regard to the contour of the colony and the growth rate. Rate of growth is considered under a separate heading. Isolate 3 produced on agar a colony margin different from that of any other isolate in that the margin was composed of radiating hyphae. In all other isolates the colony margin was opaque due to the fact that the margin was abrupt and piled above the agar surface.

It was observed that the isolates were most distinctive in their colony characteristics at a temperature of 20° or 24°.

In the standard liquid medium with 1 per cent malt extract isolates 3, 43, 69, 92, and 101 tended to produce a mat-like growth, while 24, 27, 29, 30,

77, 83, 91, and X14 produced many small distinct colonies. This difference in growth might be explained by the fact that in every case the former isolates had a higher growth rate than those giving distinct colonies.

In this same medium after 4–8 weeks, it was observed that isolates 69, 91, and 92 gave a culture which, with the application of good suction, filtered very slowly through Schleicher and Schuell filter papers No. 589. With isolates 3, 6, 27, 30, 43, and X14 this difficulty was not encountered.

It was thought that the cause of the slow filtering might be the presence of a gum-like substance produced in the culture. Attempts to precipitate in alcohol such a substance from the filtrates of the slow-filtering cultures were unsuccessful. These filtrates had exactly the same viscosity as those that filtered easily, when tested with a pipette delivering 10 cc. of water in $1\frac{1}{2}$ minutes.

Spore Production

Production of Conidia in Relation to Age of Culture. Lima-bean agar in uniform tubes and with a uniform slant was thoroughly seeded by pouring a concentrated spore suspension of each isolate used from tube to tube. The cultures were placed at 20°. At intervals graduated from 2 to 10 days, 2 tubes of each isolate were examined for spore production. This was done by introducing 3 cc. of water into each tube and thoroughly scraping the spores into suspension. After suitable dilution of the suspension, the number of spores per low power field of the microscope was counted in drops of the suspension on a slide. The drops were transferred with a wire loop 4 mm. in diameter. The average of 10 counts made near the center of each of 10 drops from the duplicate tubes was used for each determination.

Considerable variation was found between the isolates in the number of spores produced in the 2 series run. Six of the 9 isolates used in one series are represented in figure 1. Germination counts were made of the spores on 1.7 per cent agar plates after 48 hours at 20°. Spores of the various isolates gave from 92–98 per cent germination on the 8th day after seeding, from 69–98 per cent on the 32nd day, and from 40–98 per cent on the 42nd day. Isolates 30 and X14 gave the highest germination and isolates 83 and 27 the lowest.

Production of Conidia in Relation to Temperature. Lima-bean-agar slant cultures were prepared as described for the study of conidial production in relation to age of culture. The cultures were incubated at 20° for 6 days and placed in duplicate at the following temperatures: 4, 8, 12, 16, 20, 24, 28, and 30°. Three weeks from the time of seeding the cultures were examined, as described previously, for the number of spores produced. The 18 isolates tested in 3 series varied considerably in number of spores produced and in temperature relations for spore production. Six of the isolates varying most are represented in figure 2.

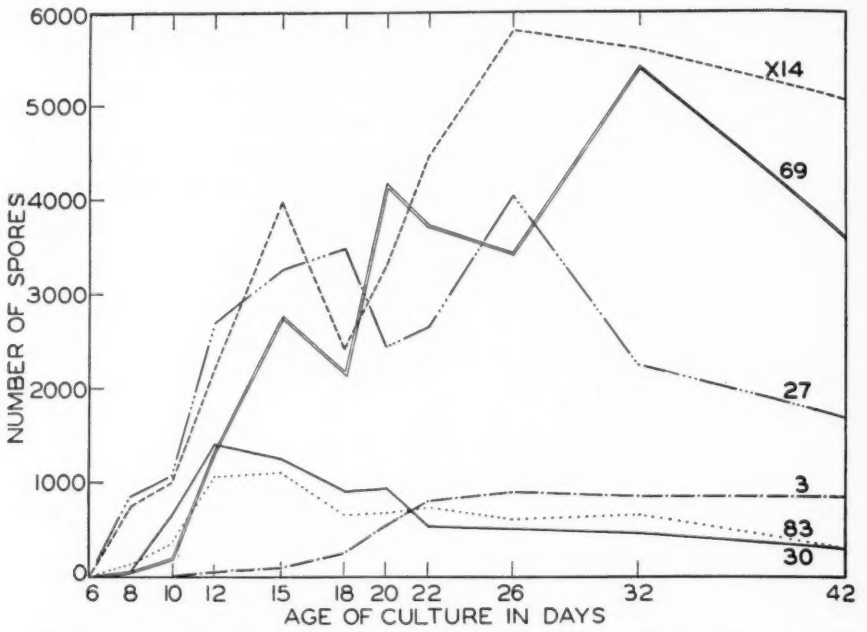

FIG. 1. The age of Lima-bean-agar cultures in relation to the number of conidia produced at 20° by 6 isolates of *C. hiemalis*.

Several isolates, including 1, 52, and X25, sporulated much more sparingly in culture than isolate 3 (Fig. 2).

Production of Microconidia. Since Craigie (7) demonstrated the functioning of the spermatia in certain rusts, the attention of mycologists has been drawn to similar bodies, microconidia, which are produced by so many of the ascomycetes. Tulasne, the first to describe these bodies, believed that sometime they would be demonstrated to be pollen-like in function. In a few instances microconidia have been shown to have a sexual function. Drayton (11) found two self-sterile races of *Sclerotinia gladioli* (Massey) Drayton, either of which, when "spermatized" by the microconidia of the other race, were interfertile and produced apothecia. Dowding (10) demonstrated the functioning of "oidia" in *Ascobolus stercorarius* (Bull.) Schroet.

Higgins (18) noticed the occurrence of microconidia on leaves parasitized by *Coccomyces hiemalis*. Keitt, while working with cultures of the fungus, commonly found microconidia produced in old Lima-bean-agar cultures. These bodies were not so commonly found by the writer, even when older Lima-bean-agar cultures developed at 20° were kept at 8–12° for several months.

In connection with studies on the relation of temperature to the growth

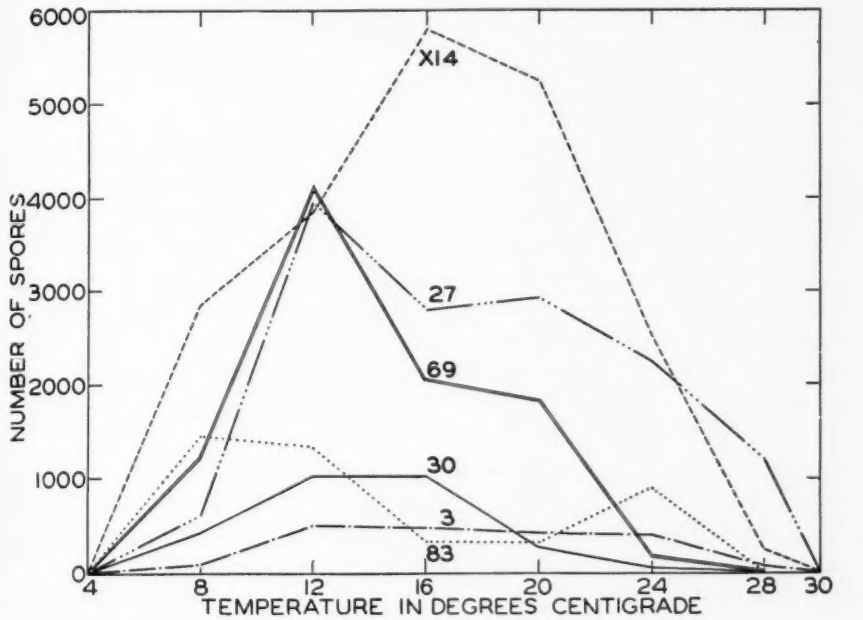

FIG. 2. Temperature in relation to spore production of 6 isolates of *C. hiemalis* grown on Lima-bean agar for 21 days. Cultures were incubated at 20° for the first 6 days.

of colonies on the standard agar plus 1 per cent malt extract, examinations were made at different periods for the occurrence of microconidia on colonies of the various isolates at the different temperatures. It was found that 3- to 5-week-old colonies of isolates 3, 30, 77, and X14 produced microconidia abundantly. Occasionally, a few microconidia were observed from isolates 83 and 91. More than 16 other isolates were never found to produce them under the stated conditions. These bodies were produced at temperatures from 8°–24°, 4° being too low and 28° too high for their production under the conditions of the experiment. Production of microconidia could not be correlated with any other characteristics of the isolates.

An attempt was then made to find microconidia on leaves parasitized by several isolates, including those producing these bodies in culture. Leaves bearing one- or two-month-old lesions were picked, washed, and put in Petri-dish moist chambers. Three days later they were again washed to remove the conidial horns and then replaced in the Petri dishes. They were kept at 12° for 3 days and then at 8° for 8 days, and examined for the production of microconidia. Lesions of all 12 isolates tested were found to contain few to many microconidia. Conidia were usually found in abundance also.

The microconidia of the various isolates varied little in size. Their

lengths ranged from 3.6 to 6.4 microns with an average of 4.9 and the width varied from 1.8 to 2.2 microns.

Germination of Conidia

Spores from 2- to 3-week-old Lima-bean-agar slant cultures were germinated at 20° for 48 hours. Ordinarily, spores germinate 100 per cent on 1.7 per cent agar plates with, or without, nutrient salts. Germination in drops of pure water on slides or in Van Tieghem cells is poor and erratic.

Lack of oxygen completely inhibits germination. Carbon dioxide given off by bits of crushed cherry leaves in Van Tieghem cells or Petri-dish moist chambers is toxic to the spores, but, if this gas is absorbed by the introduction of vessels containing barium hydroxide solution, normal germination results.

In the studies on the source of carbon it was desired to add the sugars after the basic liquid medium had been seeded and initial growth had taken place. Therefore the basic liquid medium in 250 cc. flasks was autoclaved separately in 2 series and seeded with conidia before the sources of carbon were added. It was found that little or no germination took place in the absence of the carbon source, whereas, in the full nutrient solution, germination proceeded normally.

The effect of sugars and some other compounds on germination was then studied in Van Tieghem cells, using conidia of isolates 69 and X14. The results of two series are presented in table 1. The drop of liquid placed in the bottom of each cell corresponded to the liquid in which the spores were suspended. About 100 spores per drop was found suitable. Since the two isolates gave similar results, the average of their germination counts is given.

The lack of germination in the absence of sugars, which was observed in 50 cc. of medium in a flask, could not be duplicated in Van Tieghem cells. Since the spores in the Erlenmeyer flask were at the bottom of the liquid, and in the cell were in contact with the air at the bottom of a drop, the discrepancy in results is probably due to the difference in the availability of oxygen.

The results of the germination tests shown in table 1 indicate that spores germinate better in an osmotic concentration as found in a balanced nutrient medium, that peptone and malt extract promote growth, that NaCl and aspartic acid are somewhat toxic, and that glycerine supports less germination than sucrose, under the condition of the experiments.

In the studies on the relation of hydrogen-ion concentration to germination, the medium used consisted of 1.7 per cent agar, 0.4 per cent KH_2PO_4, and 0.05 per cent $MgSO_4$ in distilled water. The hydrogen-ion concentration was adjusted by the addition of 1/5 normal HCl or 1/5 normal NaOH

TABLE 1.—*The effect of various organic and inorganic materials on germination of conidia of Coccomyces hiemalis in Van Tieghem cells*

Germination medium[a]	Germination after 48 hours	
	Average	Average length of germ tubes
	Per cent	*Microns*
Distilled water	23	19
City water	42	34
Solution of NaCl, 0.85 per cent[c]	28	15
NaCl, 0.85 per cent + sucrose, 2 per cent[c]	24	15
Pure sucrose, 2 per cent	77	43
Pure glycerine, 2 per cent[d]	28	20
Salt solution[b]	86	57
2 per cent sucrose + salt solution	92	61
2 per cent glycerine + salt solution	87	68
Peptone, 0.5 per cent	100	120
Glutamic acid, 0.5 per cent[c]	75	38
Glycine, 0.5 per cent[c]	42	26
Aspartic acid, 0.5 per cent[d]	0	0
Liquid malt extract, 2 per cent[c]	100	136

[a] Adjusted to pH 5.5 with $\frac{N}{5}$ NaOH and autoclaved 15 minutes.

[b] The salt solution containing 0.4 per cent KH_2PO_4 and 0.05 per cent $MgSO_4$.

[c] Used in one series only.

[d] Spores in these media appeared vacuolate.

and determined both before and at the end of the 48-hour period with the quinhydrone electrode. Determinations of pH were made on the cold agar. A drop of the spore suspension containing about 200 spores was placed on the agar surface. Agar plates were in duplicate, each plate being used for spore suspensions of 4–6 isolates.

At the end of the 48-hour period of germination, 4–6 drops of concentrated commercial (40 per cent) Formalin was added to each plate. By fixing the germinating spores in this manner, a large series could be run, and more accurate results obtained. To get the percentage germination at least 50 spores were counted in each drop and for the length of germ tubes at least 25 spores, giving at least 100 and 50 spores, respectively, for any one reading. The method of taking the readings follows: in each low-power field of the microscope the number of germinated spores and of ungerminated spores was recorded and the length of the germ tubes of each spore in the field was taken by means of a calibrated ocular micrometer. The per cent of germinated spores was calculated on the basis of the total number of spores observed and the length of germ tubes was based on the number of germinated spores used for the measurements.

The results of 3 series showed that the 6 isolates used had the same range of pH for spore germination, from pH 2.9 to 8.5. Spores germinated 100 per cent from about pH 4 to about pH 7. The curves for the average length of germ tube, however, had a distinct maximum at pH 5.3 to 5.6. The isolates showed some variability in the average length of germ tubes. The curves for the average length of germ tubes of 5 isolates of one series are plotted in figure 3.

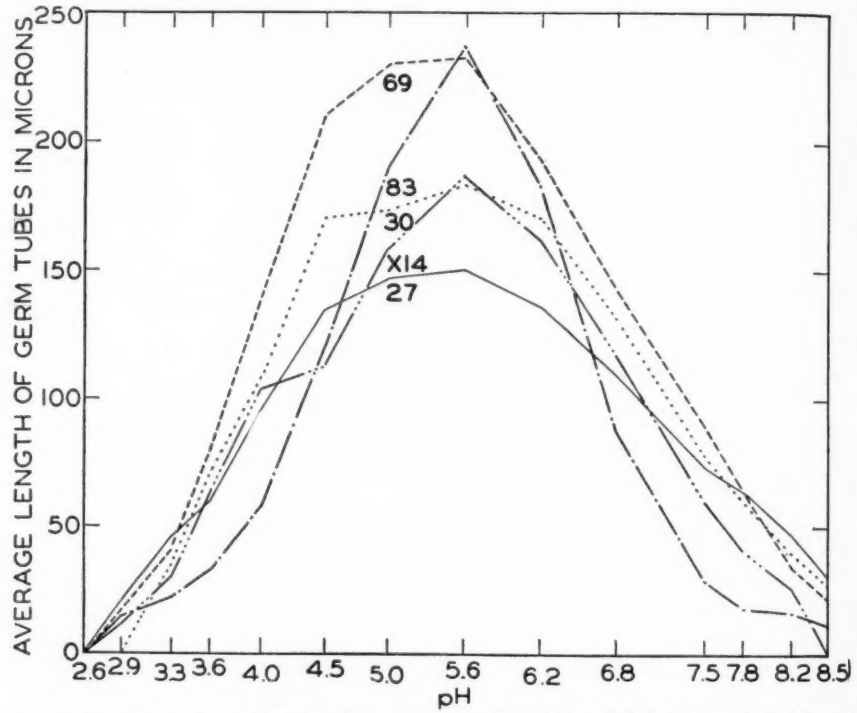

FIG. 3. The average length of germ tubes of conidia of 5 isolates of *C. hiemalis* germinated at 20° C. on agar buffered at various hydrogen-ion concentrations.

Growth Rate

Six isolates were grown in two series in a liquid medium for 12 weeks. The dry weight of the mycelium, total acidity, and pH were taken of duplicate flasks of each isolate every week. The medium contained:

KH_2PO_4	0.2 per cent	Trommer's malt extract	1 per cent
Peptone	0.1 per cent	Dextrose	2 per cent
$MGSO_4$	0.05 per cent	Initial pH about 6	

The hydrogen-ion concentration was obtained by the use of the quinhydrone electrode. The amount of growth differed between some of the isolates, but the type of curve was essentially the same for all (Fig. 4).

The change in hydrogen-ion concentration produced by the several isolates was not proportional to the corresponding amount of growth.

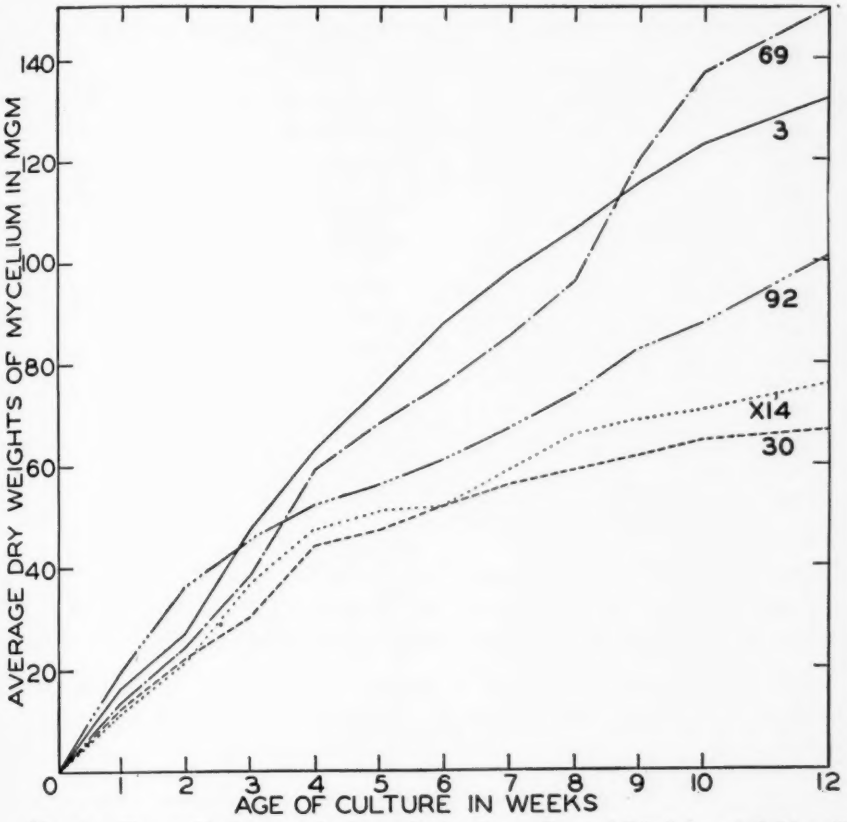

FIG. 4. The weekly increase in dry weights of mycelium of 5 isolates of *C. hiemalis* grown on a liquid medium. Average of 2 series.

The variation in acid production was considerable (Fig. 5). Change in pH was about proportional to the amount of acid produced. The original medium was plus 11.2 Fuller's scale. Isolate 92 changed it in 12 weeks to plus 13.2 and isolate 3 to plus 17.4, other isolates being intermediate in acid production.

The filtrates from the 6-weeks-old cultures of one series were sterilized and reseeded, one of the duplicate filtrates with isolate 30 and the other with isolate 69. The results taken at the end of the 12-week period clearly showed that with both isolates, 30 and 69, the mycelial dry weights of the second seeding were inversely proportional to those of the first seeding (Table 2). This would indicate that the cause for the variation in the amount of growth from the second seeding was the difference in the amount

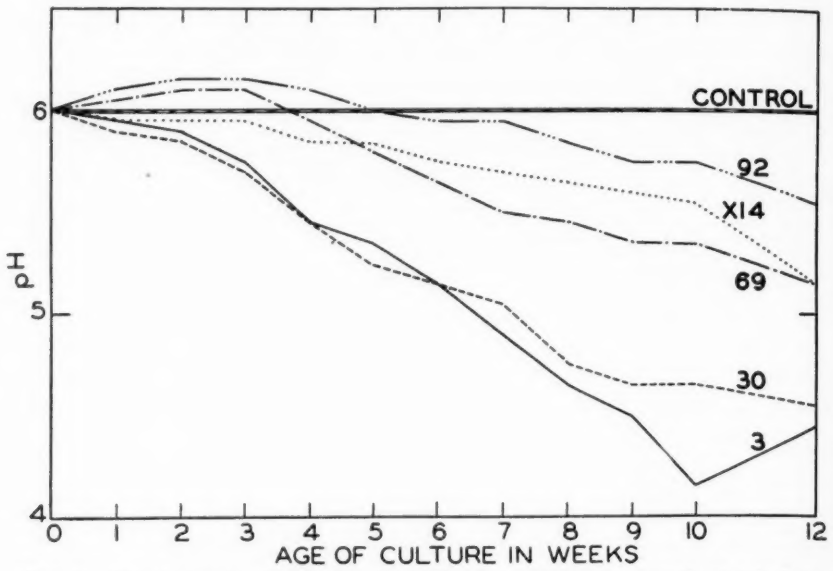

FIG. 5. The change in pH of a liquid medium caused by the growth of 5 isolates of *C. hiemalis*. Average of 2 series.

of nutritive material rather than the presence of staling products. The presence of a toxic material produced in greater amounts by one of the isolates than another was not indicated by these results. Staling products

TABLE 2.—*Results of experiment on growing cultures in a liquid medium partially depleted of nutrients by the growth of other cultures of Coccomyces hiemalis*

Flask[a]	Seeded on 11–23–33		Reseeded on 1–5–34	
	Isolate	Weight of mycelium on 1–5–34	Isolate	Weight of mycelium on 2–17–34
No.	*No.*	*Mgm.*	*No.*	*Mgm.*
1a	43	80	30	14
1b	43	76	69	29
2a	30	47	30	24
2b	30	51	69	34
3a	69	89	30	13
3b	69	97	69	30
4a	92	71	30	16
4b	92	69	69	33
5a	X14	55	30	19
5b	X14	59	69	38

[a] The medium contained in each flask was filtered and autoclaved on 1–5–34 preparatory to reseeding.

may have been present in small amounts or may have been destroyed by autoclaving.

Colonies of the same isolate or of different isolates grown side by side on agar grew equally in all directions and did not show the inhibition of growth in the direction of neighboring colonies so apparent with some fungi. An increase in the number of colonies per plate reduced their size after about 4 weeks. Two colonies of a certain isolate on one agar plate had diameters after 6 weeks twice as great as those of the same isolate on another plate where 12 colonies were growing.

Temperature Relations

The standard agar with 1 per cent malt extract was used in this study. After seeding, the plate cultures were put at 20° for 7 days, when the colonies were measured by taking the average of the longest and shortest diameters. The plates were then placed in 4 replications at 4, 8, 12, 16, 20, 24, 28, and 32°. After 6 weeks from the time of the first measurement, the colonies were again measured and the difference between the first and the second measurements was taken as the amount of growth at the stated temperatures. In preliminary series, measurements taken every week showed the isolates to have about the same relative growth rate from week to week.

The isolates gave similar growth curves, in that their maxima lay between 20° and 24° with a sharp falling off at higher temperatures, no growth taking place at 32°, and a gradual decline in growth at lower temperatures, a small amount occurring at 4°. The relative amounts of growth varied

TABLE 3.—*Effect of temperature on the diameter of colonies of 13 isolates of Coccomyces hiemalis grown on the standard agar with 1 per cent malt extract*

Isolate number	Diameter[a] of colony in mm. after 6 weeks at the stated temperatures							
	4	8	12	16	20	24	28	32
1	3.9	5.3	8.3	12.5	14.4	14.4	9.2	0
3	3.6	6.1	9.5	12.8	17.4	15.6	5.8	0
24	2.9	3.2	4.0	5.8	8.8	10.1	5.9	0
27	2.6	4.1	7.0	8.9	10.4	11.2	6.6	0
29	2.6	3.8	5.6	8.5	10.6	11.6	4.7	0
30	3.8	5.7	8.2	10.3	11.4	11.4	5.2	0
66	3.3	5.6	6.7	8.3	9.8	8.0	5.5	0
69	2.9	5.0	8.6	12.7	16.9	16.3	6.0	0
77	3.3	4.8	8.7	10.4	10.4	10.8	6.4	0
83	2.2	4.1	6.6	7.7	9.2	9.3	6.7	0
X14	3.2	4.8	6.0	8.0	9.2	8.8	7.3	0
91	4.3	5.8	8.5	10.4	15.2	14.0	10.2	0
92	2.0	3.7	5.0	8.0	13.7	14.0	6.3	0

[a] Each diameter is the average of 8 readings taken in 2 series.

considerably between the isolates (Table 3). Isolates 1, 3, 69, and 91 produced colonies at the 3 temperatures, 16, 20, and 24°, with diameters from 51 to 89 per cent greater than the diameters of colonies produced by isolates 24, 66, 83, and X14.

Light Relations

A preliminary survey of the effect of ultraviolet radiation on the sporulation of several isolates of *Coccomyces hiemalis* in culture is reported here.

The action of ultraviolet radiation on fungi has been studied more than that of any other portion of the spectrum, and it is to these rays that fungi are apparently most susceptible. Several investigators have reported 2 effects of ultraviolet radiation on fungi: inhibition or lethal action, and stimulation of fruiting body formation. The former action is reported by Johnson (20) for several fungi showing a suppression of growth from, and adaptive reactions against, the injurious action of ultraviolet radiation. Stevens (45) found a stimulation of the production of fruiting bodies in *Glomerella cingulata* (Atkin.) S. & S. when cultures were exposed to the full range of ultraviolet light for a period as short as 4 seconds. An increase in the sporulation of *Macrosporium tomato* and of *Fusarium cepae* was reported by Ramsey and Bailey (34) to be induced by ultraviolet radiation.

Malt-extract-agar plates, seeded over the entire surface with concentrated spore suspensions, were incubated at 20° for 6 days. The plates were then exposed to the full range of ultraviolet light from a quartz mercury-vapor lamp at a distance of 66 cm. for periods from ½ to 30 minutes. Since no filters were used, the light included some of the visible and infrared range as well as the ultraviolet. Cultures exposed for 2 weeks to diffuse daylight showed no difference in color or spore production from those kept in total darkness. The heat effect of the lamp at 66 cm. was considered negligible.

The effect of the infrared range on cultures of *Coccomyces hiemalis* was not studied. No report of a positive action of this range of wave length on fungi was found in the literature. It is inferred, therefore, that the effect of irradiation reported here was due to the ultraviolet range of radiation.

Four to six days after irradiation, a disc of the agar plate culture was cut out with a large cork borer and placed in a standard amount of water. The spores were thoroughly scraped off into suspension and, after suitable dilution, the number of spores per low power field in 6–8 drops on a slide was counted. Only one field in the center of each drop was used. This was repeated with a second disc of the culture. The average number of spores per low power field was taken as the relative value of sporulation.

Four isolates were used in each of the 3 series made. Isolates 83, 91, 101,

and X14 were repeated in the third series. The results in table 4 give evidence that treatments between ½ and 5 minutes stimulated the cultures to greater sporulation and that longer treatments showed a more or less decided inhibitory effect. Isolates 6, 91, and 101 were stimulated by the shorter period of treatment, whereas cultures 3, 27, 69, 83, and X14 were stimulated only slightly. Treatments of only 3 minutes or more inhibited sporulation of isolate 83, while it took treatments of 10 minutes or more to reduce the sporulation of isolate 101. Treatments of 10, 20, and 30 minutes collapsed the aerial mycelium of all isolates.

TABLE 4.—*Effect of ultraviolet radiation on the sporulation of 8 isolates of Coccomyces hiemalis on 2.5 per cent malt-extract-agar plates*

Isolate number	Average number of conidia per low power field							
	Untreated	Treated with ultraviolet radiation for stated number of minutes						
		½	1	3	5	10	20	30
83[a]	84	109	120	51	43	8	8	9
91[a]	10	81	139	83	68	77	49	47
101[a]	16	64	134	202	361	5	6	3
X14[a]	279	443	244	116	113	87	92	72
3	23	58	.....	43	47	25	5	2
6	102	262	274	204	75	62	68	51
27	129	51	174	224	47	31	17	17
69	21	52	40	35	30	8	16	.....

[a] The results represent the averages of 2 series.

Source of Nitrogen

The sources of nitrogen were used in 0.4 per cent concentration in combination with the basic medium:

KH_2PO_4 ..... 0.4 per cent
$MgSO_4$ ..... 0.05 per cent
Dextrose ..... 2.0 per cent
pH 5.5

The following sources were tested in both the liquid and agar basic medium: peptone, asparagin, aspartic acid, glutamic acid, glycine, ammonium nitrate, potassium nitrate, calcium nitrate, potassium nitrite and urea. In addition, ammonium sulfate, ammonium lactate, and ammonium tartrate were tested in the agar medium.

Cultures were grown at 20° for 6 weeks. Seven isolates, tested in duplicate in 3 series, using 50 cc. of the liquid medium in 250 cc. Erlenmeyer flasks, gave good growth (dry weights of mycelium from 40 to 95 mg.) with peptone. On none of the other sources was good growth obtained. Of

these, glutamic acid gave the best (7–14 mg.). Urea was rather toxic and potassium nitrite prevented germination. With all the sources of nitrogen except peptone, urea, and potassium nitrite, the spores germinated and grew for 1–2 weeks, after which very little, if any, growth took place. The dry weights of mycelium in these cases varied from 2–6 mg. On the basic medium, used alone as a control, germination took place and dry weights of mycelium from 0–2.5 mg. were obtained. Acetamide and alanine used in one series did not support growth of 5 isolates.

The effect of the various sources of nitrogen on the growth of 18 isolates was tested in several series in the basic medium made solid by the addition of 1.7 per cent agar. The agar was not washed. Potassium nitrite inhibited germination of the spores used to seed the plate. Ammonium lactate and urea allowed little or no growth. On all of the other sources the diameters of the colonies produced by any one isolate were very comparable. Even on the basic medium used alone the colony diameters approached those of colonies grown on the peptone medium. Only on the peptone medium, however, did the isolates produce their characteristic colonies. On all the other sources of nitrogen the colonies were thin, with diffuse mycelial growth and no characteristic piling up of the colony above the agar surface.

On the peptone agar and on malt-extract agar, certain isolates produced a pigment that blackened the medium. This black pigment, produced only on peptone or malt extract, may indicate the production of tyrosinase by the fungus, giving a melanin pigment by the oxidation of tyrosin. With any of the other sources of nitrogen, none of the isolates blackened the substrate, nor did they develop their characteristic colors. Some of the isolates produced light shades of pink, yellow or brown on the poor sources of nitrogen. Colonies grown on ammonium lactate, potassium nitrate, and control agars were colorless.

There was little difference between the various isolates tested in regard to their nitrogen requirements, but they showed the usual variation in their relative amounts of growth.

In this study, malt extract used as a source of nitrogen gave results similar to those of peptone.

In preliminary tests, it was found that the addition of small amounts of malt extract to the media, containing peptone or an amino acid, increased the dry weights of mycelium markedly. To test this effect further, 5 isolates were grown in the basic liquid medium, with and without the addition of 0.04 per cent malt extract, and containing peptone, glumatic acid, aspartic acid, glycine, ammonium nitrate, potassium nitrate, or calcium nitrate.

The results of 2 series showed that the presence of the malt extract increased the availability of peptone, glumatic acid, and aspartic acid. In

the case of each of the 5 isolates, the increase in dry weights of mycelium produced on these 3 nitrogen sources by the addition of the malt extract was more, by at least 90 per cent, than could be accounted for by the growth that took place on the basic medium plus 0.04 per cent malt extract alone. In fact, 2 isolates gave more growth on these 2 amino acids than on peptone.

In the case of glycine, ammonium nitrate, potassium nitrate and calcium nitrate, malt extract did not, apparently, make them more available, for the dry weights of mycelium were about the same as those produced by the 5 isolates on the basic medium plus 0.04 per cent malt extract, used as control.

The effect of the addition of 1 per cent yeast extract, 1 per cent malt extract, and 10 per cent sour cherry leaf extract to the standard liquid medium was tested in duplicate, using isolates 30, 91 and X14. The leaf extract was made by steeping 100 grams of Montmorency cherry leaves in a liter of distilled water at 60° for 5 hours. The results show that all 3 of the extracts just about doubled the dry weights of the mycelium in 6 weeks, the leaf extract being about $\frac{2}{3}$ as effective as the other 2 extracts.

The effect of a small amount of malt extract in making some of the amino acids more available is striking. The assumption is that the malt extract contains growth-promoting substances in the category of hormones or vitamins, or rare elements, or some unknown accessory growth factor. Spores will germinate and growth will continue for the first week or two in the absence of the growth promotor, but its presence is apparently necessary for any considerable growth. The spores apparently contain enough of this substance to allow for the initial growth in a medium lacking the growth promotor.

The Parke, Davis and Company bacteriologic peptone, used in this investigation, may contain an amount of the growth promotor, insufficient for best growth of the fungus, for the addition of malt extract, yeast extract, or leaf extract to the peptone medium greatly increases the dry weights of mycelium obtained. The presence of a small amount of a growth stimulator in this peptone may explain why it supports growth where amino acids do not. If this is true, then peptone, without this growth stimulator, might be as poor a source of nitrogen as the amino acids used. The fact that the glutamic acid used in this study was of the "practical" grade, *i.e.*, rather impure, may explain why this amino acid allowed more growth than aspartic acid or glycine which were of C.P. grade.

Source of Carbon

Sixteen isolates were tested in several series for variability in their physiological requirements in relation to the sources of carbon on solid and liquid media. The basic medium consisted of:

Peptone	0.4 per cent	KH_2PO_4	0.4 per cent
$MgSO_4$	0.05 per cent	pH 5.5	

The sources of carbon, added in 2 per cent concentration, were sodium citrate, glycerine, arabinose, l-xylose, dextrose, galactose, levulose, lactose, maltose, sucrose, mannite, salacin, raffinose, inulin, dextrin.

There were no consistent differences between the isolates in regard to source of carbon utilization, all isolates tested showing nearly the same growth relation on any one source of carbon. The only consistent variability noted was in the relative amounts of growth produced by the different isolates.

The sources of carbon are ranked, according to the amount of growth they supported with the various isolates on the liquid and the solid medium, in classes, as follows: good growth on dextrin, dextrose, galactose, lactose, levulose, maltose, mannite, raffinose, and sucrose; fair growth on arabinose and inulin; poor growth on glycerine and salacin. Sodium citrate and l-xylose produced no growth and prevented spore germination.

The results with 3 isolates in one liquid medium series are given in table 5. Isolate 69 is representative of the faster growing isolates and X14 of the slower growing ones.

When, instead of being autoclaved separately, levulose was sterilized with the liquid medium, very poor growth resulted. This effect was not observed when levulose was sterilized with the agar medium or added to the liquid medium after autoclaving.

With most of the sources of carbon the growth of the isolates reduced the pH of the liquid medium considerably. However, with glycerine and especially with inulin the isolates raised the pH of the medium and also blackened it. This may indicate that with these poorer sources of carbon some of the peptone was broken down and utilized as a source of carbon, liberating ammonia and reducing the hydrogen-ion concentration.

MORPHOLOGICAL STUDIES

Considerable variation was found in the length of conidia between some of the isolates of *Coccomyces hiemalis*. Conidia were obtained from 3-week-old Lima-bean-agar cultures and were measured under the high-power magnification, $\times 467$, of the microscope, equipped with a camera lucida. By placing a stage micrometer under the microscope, a scale was drawn on a piece of paper. By replacing the stage micrometer with the conidia, their lengths in microns were obtained by superimposing the scale upon their image. The use of a mechanical stage guarded against replicate measurements of any spore. The measurements were made to the nearest 5-micron interval. The conidia were mounted in lacto-phenol. A small amount of fast green or safranin dissolved in the mounting fluid proved helpful.

TABLE 5.—*The effect of various sources of carbon on the dry weights of mycelium and final pH of cultures grown for 6 weeks at 20° in liquid media with an original pH of 5.5*

Sources of carbon	Isolate 69		Isolate 83		Isolate X14	
	Average dry weights of mycelium	Final pH	Average dry weights of mycelium	Final pH	Average dry weights of mycelium	Final pH
	Mgm.		*Mgm.*		*Mgm.*	
Control	12	6.1	6	5.9	5	5.8
Arabinose	45	5.5	29	5.9	12	5.4
Dextrose	57	5.4	47	5.6	27	4.1
Dextrin	59	5.3	44	5.6	38	4.5
Galactose	46	4.7	35	5.4	29	4.5
Glycerine	29	6.3	25	6.1	14	6.0
Inulin	36	6.6	32	6.3	15	6.3
Lactose	46	4.5	40	5.5	32	4.6
Levulose	56	5.3	43	5.4	29	4.5
Maltose	53	5.3	40	5.3	33	4.2
Mannite	57	4.9	41	5.3	28	4.3
Raffinose	46	5.0	38	5.4	33	3.9
Salicin	21	4.7	20	5.5	22	4.5
Sodium citrate	0	5.2	0	5.3	0	5.2
Sucrose	50	4.9	50	5.3	23	5.1
l-Xylose	0	5.5	0	5.5	0	5.6

Many of the spores were more or less curved. These were measured by taking the length of an imaginary median line drawn through the conidium from end to end. This was done by comparing the curved spore with one of several calibrated lines, drawn on a paper, having curves corresponding to those of the spores.

Length of Conidia in Culture

The distribution in length of 500 spores each of 6 isolates in culture is shown in figure 6, A. These isolates represent the extreme of variation in conidial length as found in pure culture. The curves for isolates 69 and 91 with modes at 50 and 60 microns, respectively, are representative of the curves for 5 other isolates studied.

The average length of spore for each isolate corresponds closely with the modal length of its curve. The average spore length in microns for these 6 isolates is 45.0 for No. 30, 49.0 for No. 3, 51.2 for No. 91, 55.8 for No. 69, 67.5 for No. X14, and 78.9 for No. 83. Ninety-three per cent of the spores of isolate 30 are shorter than 60 microns and 94 per cent of isolate 83 are longer.

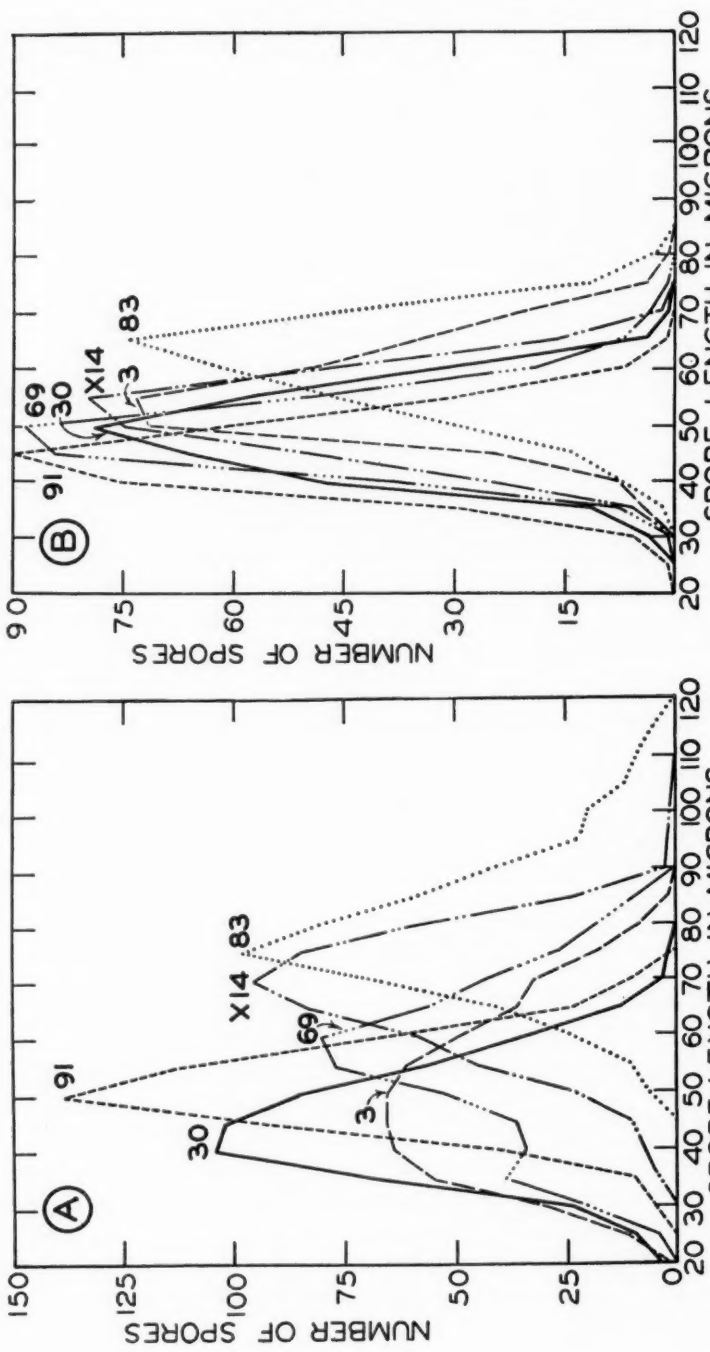

FIG. 6. The distribution in length of conidia of 6 isolates of *C. hiemalis* using (A) 500 spores of each isolate from Lima-bean-agar cultures, and (B) 300 spores of each isolate from Montmorency sour cherry leaves.

Length of Conidia on Several Hosts

Conidia of these several isolates when produced on Montmorency cherry (*Prunus cerasus* L.) leaves were found to have lengths different from those found in culture. Samples of spores to be measured were obtained from comparable leaves that were inoculated on the same date and whose lesions were 2 weeks old, 300 conidia of each isolate being measured.

The distribution of spore length for the same 6 isolates when conidia were obtained from leaves is represented in figure 6, B. The isolates varied much less in length of conidia when the latter were obtained from Montmorency cherry, and these conidia were in general shorter and more uniform in length than they were from culture. The average spore length in microns for the 6 isolates is 44.9 for No. 91, 48.8 for No. 30, 48.9 for No. 69, 51.8 for No. X14, 55.6 for No. 3, and 60.5 for No. 83.

Higgins (18) gave the range in length of conidia of *Coccomyces hiemalis* to be from 45–65 microns. The writer found the conidia of isolate 91 from sour cherry leaves to be from 25–65 microns in length and the length of conidia of isolate 83 from the same source to be from 35–80 microns. The range in length of conidia from culture is much greater (Fig. 6, A).

Little variation was found between the isolates in the number of septa per spore. The per cent of conidia from the host having one septum varied from 84 to 100. As many as 16 per cent of them had 2 septa, and less than 1 per cent had no septa. Lima bean agar cultures, on the other hand, differed in having 3 to 8 per cent of non-septate, 3 to 10 per cent of 2-septate, and an occasional 3-septate conidium.

A limited study of the width of conidia under oil-immersion objective indicated but slight variation in this regard between the isolates. The maximum diameter of conidia from culture or host varied from 1.5 to 3.4 microns with an average of 2.0–2.7 for the different isolates. Higgins reported the width to be from 2.5–4 microns.

The effect of other species of Prunus on the size of conidia was tested with 4 isolates, 200–300 spores being measured in each case. The average length of conidia produced on *P. pennsylvanica* L. was for isolate 30, 48.4 microns; for isolate 69, 45 microns; for isolate 83, 58.3 microns; for isolate X14, 54 microns. These averages compare rather closely with those obtained for spores produced on *P. cerasus*. On *P. mahaleb* L. the average length of conidium with X14 was 55.4 microns and on *P. cerasifera* Ehrh. was 44.7 microns. Sufficient spores for measurements of other isolates were not obtained from *P. cerasifera* due to the difficulty of finding sporulating lesions.

The effect of different environments on the length of spores produced on Montmorency was studied, using isolate X14. Two control chambers devised by Keitt for growing plants under controlled temperature, humidity,

and illumination were used in this study. One of these chambers was run at a temperature of 24° with a full complement of artificial light and the other at 14° with 1/3 of this light intensity. The day and night periods were 12 hours each. The humidity in both chambers varied from 75 to 90 per cent of saturation. The experimental trees were put in the chambers immediately after the moist treatment following inoculation with isolate X14. Leaves were sampled for spores 8 to 12 days after the lesions appeared and from each chamber 250 spores were measured. This was repeated with trees inoculated several days later, giving over 500 measurements from each chamber.

The spores from the low-temperature chamber varied in length from 40 to 80 microns, averaging 59.1, and those from the high-temperature chamber varied from 30 to 65 microns, averaging 47.9 microns. Further work to determine whether this variation was due to difference in temperature or in light was not feasible.

Size of Ascospores

Measurements of ascospores from sour cherry leaves obtained from different localities in Wisconsin in 1916–1917, and in 1932, showed little variation in average spore length or average width between different samples.[5]

Ascospores were shot onto plates of 1.7 per cent agar and measured as described for conidia. Their lengths varied from 25–30 to 55–65 microns, averaging between 42 and 49 microns in the various samples. The ascospores had an average width of 3.0 to 4.2 microns. Higgins' measurements for ascospores were 33–50 × 3.5–4.5 microns.

INFECTION STUDIES

Higgins (18) and Keitt (24) reported *Coccomyces hiemalis* to be pathogenic on *Prunus avium* L., *P. cerasus,* and *P. pennsylvanica;* and the latter author showed, in addition, that *P. mahaleb* was susceptible also. Keitt found that the various Prunus species exhibit a gradation of susceptibility toward *C. hiemalis* from complete susceptibility, through degrees of resistance, to immunity. He showed that this fungus sometimes infected the Damson plum (*P. insititia* L.) after a prolonged incubation period, and in addition produced aberrant lesions under certain conditions on other species and varieties of plums. These facts might suggest pathogenic variability between isolates of *C. hiemalis.*

A number of fungus pathogens in addition to the rust and smut fungi have been found to contain physiologic forms. Some of the more important will be mentioned here. Neger (32), Marchal (28), Salmon (37), and Reed (35) showed that several of the powdery mildews exhibit physi-

[5] The writer is indebted to Dr. G. W. Keitt for part of these data.

ologic forms. Eberhardt (13) described 2 pathogenically specialized races of *Albugo candida* (Pers.) Gray. Five specialized races were found in *Claviceps purpurea* (Fr.) Tul. by Stäger (40). Müller (31) made a study of *Rhytisma acerinum* (Pers.) Fr. on various maples and concluded that it consists of several specialized races. Gilbert (16) found biologic forms in *Plowrightia morbosa* (Schw.) Sacc. Gaumann (15) reported a high degree of host specialization in *Peronospora parasitica* (Pers.) de Bary. Wormald (49) found physiologic forms in *Sclerotinia cinerea* (Bon.) Wor. Palmiter (33) reported strains of different pathogenic capabilities in *Venturia inaequalis* (Cke.) Aderh.

Methods and Materials

Two-week-old Lima-bean-agar cultures and 2- to 3-week-old lesions on Montmorency sour cherry leaves were used as sources of spores for inoculations. Spores obtained from lesions were more vigorous in germination on 1.7 per cent agar in water than spores from culture. Comparative tests of the infection produced on Montmorency by spores from infected leaves and from culture, when the spore suspensions were standardized to 100 spores per low power field, showed that spores from the leaves gave as much as 50 per cent more infection than spores obtained from Lima-bean-agar cultures.

The spore suspensions of the various isolates being compared were made up to contain about 100 spores per low power field. The suspensions were sprayed on the lower surface of the leaves with an atomizer until the water ran off.

Two- and three-year-old potted trees, limited to 2–4 shoots each, were used in these studies. For any one series, trees with uniform shoot growth were selected. The inoculated trees were immediately placed in the moist chamber, devised by Keitt (25), at a temperature of 20°, where they remained for 48 hours. Subsequent to the moist treatment, the trees were kept in a greenhouse. About 3 weeks after inoculation, data were taken by counting the number of lesions in the maximally infected square inch of each leaf.

Infection Studies on Sweet and Sour Cherry

Using spores from culture and from the host, 17 isolates were tested comparatively for the amount of infection produced on Montmorency sour cherry during the spring of 1932 and of 1933. Each isolate was used in at least 3 series. Isolates 1, 52 and X25 were tested with spores from culture only. These gave either no infection or such sparse infection that sufficient inocula for reinoculations could not be obtained from the leaves. It is interesting to note that these isolates produced relatively few spores and all of them are classed according to color as flesh pink. The other

isolates used gave fair to good infection on Montmorency in all tests. The average infection per shoot varied in amount from 25 to over 150 lesions per maximally infected square inch, when spores from leaf or culture were standardized to 100 per low power field.

Data were taken 3–4 weeks after inoculation on the per cent of necrotic lesions. These lesions were 1–2 mm. in diameter, brown, and in the process of being corked out to produce "shot-holes." Greenhouse temperatures under 16° were unfavorable and over 20° favorable for the appearance of necrotic lesions. Under the conditions of the experiments, the following isolates produced, after 3–4 weeks, from 25–80 per cent necrotic lesions: 21, 27, 30, 49, 66, X2, and X12. Twelve other isolates gave from 0–30 per cent necrotic lesions: 3, 6, 17, 24, 29, 69, 76, 77, 83, 91, 92, and X14.

Prunus mahaleb gave results in a few trials similar to those of *P. cerasus*, except that infection was much less heavy and necrotic lesions did not develop.

Spores from cultures of each of 21 isolates, used on Governor Wood sweet cherry (*Prunus avium*), gave some infection with the exception of isolates 1, 11, 52, 87, and X25, which gave none. With all of the other isolates infection varied between different tests from poor to good, the variation being inconsistent.

In the 4 series, using Governor Wood, data were taken on the type of lesions produced by each isolate. It was found that some gave lesions, practically all of which had acervuli, and others produced relatively few sporulating lesions. The isolates producing sporulating lesions were 17, 20, 24, 30, 69, 77, X12, X14, and X27. The following gave the aberrant type of lesion: 3, 6, 21, 27, 66, 83, and X53. It was noticed that the lesions without acervuli were rather quickly corked out, even when very small. This corking out usually took place within 4–5 days after the appearance of the lesions and it was not usually attended by necrosis.

Prunus pennsylvanica was also used to test the pathogenicity of several isolates. This host proved unsatisfactory, due to much variation between the seedling plants. Isolates produced sporulating lesions on one plant and aberrant ones on another, both in the same series and in different series. Different isolates when inoculated on one plant gave essentially the same type of lesion. The type of lesions varied between plants from necrotic to non-necrotic and from aberrant to sporulating. Young leaves seemed to be more favorable for the production of lesions with acervuli.

Infection Studies on Other Species of Prunus

Prunus domestica L., *P. munsoniana* Wight & Hedr., *P. insititia* L., and *P. cerasifera* proved unsatisfactory as experimental material, for sparse and erratic infection resulted with the several isolates tried, the lesions

usually being aberrant. The results of one inoculation experiment did not conform with those of the next, due in part perhaps to the difference in age and condition of the leaves.

DISCUSSION

The isolates of *Coccomyces hiemalis* included in this study have been shown to vary to some extent in certain physiological reactions and in length of conidia. These variations are probably no greater in extent than would ordinarily be expected or found in single spore isolates of any fungus. Similar or greater variation has been reported by Hansen and Smith (17) for *Botrytis cinerea* Pers., by Matsumoto (29) for Rhizoctonia, by Stevens (44) for Helminthosporium, by Shear and Wood (39) for Glomerella, by Brown (4) for Fusarium, and by Mohendra and Mitra (30) for *Sphaeropsis malorum* Pk. As a result of his extensive studies of many isolates of *Botrytis cinerea,* Brierley (3) pointed out the great variation possible within a single fungus species.

The theories regarding the origin of variants in this fungus would probably include hybridization, which has been shown to be a cause of variation in some rusts, notably by Craigie (8), Stakman *et al.* (43), and Johnson *et al.* (21), and in smuts by Stakman *et al.* (42), Holton (19), and Rodenhiser (36), as well as in the ascomycete Neurospora by Dodge (9) and in Sclerotinia by Drayton (12). *Coccomyces hiemalis* has not been shown to contain self-sterile and inter-fertile thalli. However, Backus (1) in his paper on the development of the ascus in *C. hiemalis* refers to some of his unpublished data which may demonstrate the existence of heterothallism in this fungus.

Discontinuous variation occurred in many of the isolates of *Coccomyces hiemalis* in culture, usually as pie-shape sectors. Variation was manifested in colony color and contour, and in growth rate. Some of the isolates were very unstable for several generations after isolation from single ascospores. The unstable isolates were not used in these studies. Brierley (3) states that some isolates of *Botrytis cinerea* are extremely unstable in culture while others are distinguished by rarely producing discontinuous variants. "The degree of discontinuous variability, ranging from forms which seem entirely stable to forms which are ever sporting, is characteristic of the individual isolate." One of his albino isolates was stable for 13 years.

The isolates of *Coccomyces hiemalis* were found to be unsuited to the study of discontinuous variation or sectoring in culture due to the very slow growth and small size of the colonies. Growth from single spores was especially slow.

Small amounts of malt extract, yeast extract, and cherry leaf extract have been found to stimulate the growth of this fungus. The growth-pro-

moting action of these substances may be due to one or more rare elements, amino acids, peptide linkages, a hormone or vitamin, or some unknown accessory growth factor. The growth of some lower forms of life, including yeasts and other fungi, have been shown to be greatly stimulated by the addition to the nutrient medium of growth stimulators. The accessory growth factor has been variously described as "bios," "auximones," glutathione, and pantothenic acid by different workers. Buchanan and Fulmer (5) gave an excellent review of the subject of accessory growth factors. Tanner (46) discussed the "bios question" and Williams and Honn (48) the rôle of "nutrilites" in the nutrition of molds and other fungi.

Conidia of *Coccomyces hiemalis* apparently contain a small amount of a growth promotor, since in a medium containing inorganic salts and dextrose, growth proceeded for several days and then ceased. The results of the experiments indicate that some substances, containing complex protein material, greatly stimulate the growth of cultures of this fungus.

Although some isolates differed in their pathogenicity on the leaf, the results of the inoculation experiments do not indicate the existence of physiologic forms. There are many factors that play into the host-parasite relation and that modify the amount of infection and type of lesion obtained. Some of these factors are temperature, light, the age and vigor of the leaf, variation between individual host plants, and the vigor of spores used for inoculum.

SUMMARY

A number of single ascospore isolates of *Coccomyces hiemalis,* obtained from Michigan, New York, New Jersey, Pennsylvania, Oregon, and Wisconsin, were studied from the standpoint of variability in culture and on several hosts. These isolates were found to vary:

A. In colony characters, including color, contour, growth rate, and ability to blacken the substrate.

B. In spore production, some isolates producing several times more conidia than others on Lima-bean agar. Isolates were found to produce microconidia on the host, but only a few produced them in culture under the conditions studied.

C. In growth rate and acid production in a liquid medium.

D. In reaction to ultraviolet radiation, short periods, in general, stimulating sporulation and longer periods inhibiting sporulation as well as collapsing aerial mycelium.

E. In the length of conidia produced in culture and on the host. A combination of low temperature and low light intensity resulted in longer conidia from the host than were developed with a higher temperature and more light.

F. In the amount of infection and type of lesion produced on sweet and sour cherry leaves.

Conidia from cultures of several isolates were found to germinate over a range of pH from 2.9 to 8.5 with a maximum for germ-tube length at about pH 5.4.

Colonies an agar grew and produced conidia at temperatures from 4–28° C., no growth or sporulation occurring at 32°. The optimum for growth lay between 20° and 24° for all isolates, yet at these temperatures certain isolates grew almost twice as rapidly as others. The optimal temperature for spore production varied with the different isolates, some producing more at 12° and others more at 16–20° C.

Of a number of organic and inorganic sources of nitrogen tested in a liquid and a solid medium, peptone alone was found to allow good growth of the isolates. In the presence of a small amount of liquid malt extract, glutamic acid and aspartic acid also supported good growth. Malt extract, yeast extract, and cherry leaf extract acted as growth stimulators.

Many mono- and disaccharides as well as dextrin, inulin, mannite, raffinose, and arabinose supported growth in a medium, liquid or solid, containing peptone and nutrient salts. Glycerine was a poor source of carbon and sodium citrate and l-xylose were toxic.

The isolates from any one locality varied as much as those from widely different localities.

A few isolates that sporulated sparingly in culture, or that produced feebly germinating conidia, were found to be either non-pathogenic or weak pathogens on sweet and sour cherry. The existence of physiologic forms of the fungus was not indicated.

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INCUBATION PERIOD OF PEA MOSAIC IN THE APHID, MACROSIPHUM PISI[1]

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INTRODUCTION

Although aphids are known to spread a considerable number of plant-virus diseases, little attention has been given to the manner in which they carry and transmit the viruses causing these diseases. For such a study the pea aphid, *Macrosiphum pisi* Kaltenbach, offers many advantages, being a relatively large insect, easy to handle in experiments, and easy to maintain in culture. Moreover, it is the carrier of a pea mosaic that is difficult to transmit by mechanical methods.

In the transmission of several plant viruses by leaf hoppers and thrips it has been demonstrated that an interval, referred to as an incubation period, must elapse after the insects first feed on diseased plants before they are capable of infecting healthy plants. It has also been shown that these vectors retain the virus for considerable periods of time. This has led some investigators to hold the view that viruses may multiply or pass through a stage of development in insect vectors.

The evidence that plant viruses may undergo an incubation period in aphid vectors is very meager. The only instance thus far reported is for the virus of potato leaf roll in the aphid *Myzus persicae* (Sulzer). For several other viruses it has been shown that if an incubation period occurs it is extremely short. It was of particular interest, therefore, to determine whether or not transmission of pea mosaic by the pea aphid involves an incubation period of the virus in the insect. It also seemed of interest to determine whether or not the virus is retained by the insect for a considerable period of time. In cases in which leaf hoppers serve as vectors and in which an incubation period has been demonstrated, transmission is known to occur in each instance through the agency of only a single species of insect. Moreover, mechanical transmission of the diseases is difficult or has not been obtained. It was desirable, therefore, to determine whether or not pea mosaic can be transmitted by other insects than the pea aphid, and to what extent it can be transmitted by mechanical methods. The purpose of this paper is to present the results of experiments undertaken in a study of these points.

REVIEW OF LITERATURE

Taubenhaus (18) appears to have been the first to report the pea aphid as a carrier of a mosaic disease. He found that it would transmit mosaic

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in sweet peas. The pea aphid also has been reported as a carrier of mosaic diseases in peas and clover by Dickson (2), Doolittle and Jones (3), and Böning (1). It has been reported by Zaumeyer (19) as the carrier of mosaic from peas and sweet peas to beans. These studies, however, were not concerned with the mode of transmission by the pea aphid, and no reference was made regarding an incubation period of the virus in the insect.

Smith and Boncquet (15) were apparently the first to report on incubation period of a plant virus in an insect carrier. They found that the virus of curly top of beet had an incubation period of 24 hours in the beet leaf hopper. Once acquired, the virus of curly top of beet was shown by Stahl and Carsner (16) to be retained by the insect throughout its life. Severin (12) found that at high temperatures (100° F.) the minimum incubation period of curly-top virus in the beet leaf hopper might be as short as 4 hours.

An incubation period has been shown by Kunkel (9) for aster yellows in the leaf hopper, *Cicadula sexnotata* Fallen, and by Storey (17) for maize streak in the leaf hopper, *Cicadulina mbila* (Naude). It has also been shown by Samuel *et al.* (11) for spotted wilt of tomato in the thrips, *Frankliniella insularis* (Franklin), and by Linford (10) for yellow spot of pineapple in *Thrips tabaci* Lindeman. The papers of Elze (6) and Smith (13, 14) indicate that the potato leaf-roll virus undergoes an incubation period in the aphid, *Myzus persicae*.

On the other hand, the viruses of certain diseases, such as cucumber mosaic and yellow dwarf of onions, do not require an incubation period in aphids known to serve as vectors. Doolittle and Walker (4) found, in studies on the transmission of cucumber mosaic by the melon aphid, *Aphis gossypii* Glover, that the insects acquired the virus in a period of 5 minutes on a diseased plant and were able to infect healthy plants within an equally brief period of feeding. They lost the ability to infect healthy plants soon after being removed from diseased plants. Hoggan (7) states that, in the transmission of cucumber-mosaic virus to tobacco, the peach aphid, *Myzus persicae*, acquired and transmitted the virus in a total period of not more than 30 minutes, and that it lost its infectivity after feeding for a 2-hour period on a healthy plant. Drake *et al.* (5) found that 50 different species of aphids served as carriers of yellow dwarf of onions. If the virus requires an incubation period in the insects reported as carriers, this period is extremely short. Furthermore, the aphids lost the virus in a short period of time after removal from diseased plants.

MATERIALS AND METHODS

The mosaic disease used in these experiments was obtained from *Vicia faba* L. plants growing in Westchester County, New York. The disease is

referred to in this paper as pea mosaic. In order that material might be available at all times, the disease was maintained continuously in a greenhouse by successive transfers to fresh plants.

Colonies of the pea aphid were reared on healthy *Vicia faba* plants in screened cages. Inoculations were made by transferring aphids that had fed on diseased plants to small healthy plants under screened cages or lantern chimneys. The cages and chimneys used were similar to those described by Kunkel (9).

In all of the experiments equal numbers of exposed and control plants were grown. Except for the differences noted below, the controls were given a treatment similar to that afforded the plants under test. Aphids that had been reared on healthy plants were divided into two lots. One lot was placed on diseased plants and the other was left on healthy plants. Aphids of the lot that had fed on diseased plants were then used to inoculate plants under test, while the control plants were exposed to aphids from the lot that had fed only on healthy plants. At the end of an exposure period the aphids were removed and the plants fumigated with Nicofume before return to a greenhouse.

HOST RANGE AND DESCRIPTION OF THE DISEASE

Pea mosaic was transmitted by means of the pea aphid from *Vicia faba* to garden pea, *Pisum sativum* L., field pea, *P. sativum* var. *arvense* Poir, sweet pea, *Lathyrus odoratus* L., and crimson clover, *Trifolium incarnatum* L. By the same method it was transmitted back from each of these plants to *V. faba*. Attempts to transmit the disease to red clover by means of the pea aphid and by mechanical methods were not successful. Several mosaic diseases have been observed in *V. faba*, peas, and crimson clover. A description of the disease with which this paper is concerned is, therefore, presented here.

Diseased plants of *Vicia faba* are characterized by a conspicuous mosaic pattern that appears in the leaves. This pattern, which takes the form of a spotting rather than of a mottling, is illustrated in figure 1, A. The spots are somewhat irregular in shape and quite variable in size. They tend to appear along the veins and in some plants expand into broad stripes. Symptoms of the disease have occasionally been observed 5 days after inoculation. Usually they appear in from 6 to 14 days. Some stunting has been observed in diseased plants, though this is usually not severe.

When transmitted to the Telephone variety of garden pea, the disease produces a wrinkling of the leaves with some stunting of the plant. Yellowish spots that appear on the leaves later become white and somewhat transparent. Enations, similar to those described by Jensen (8) as resulting from tobacco-mosaic infection in certain species of Nicotiana L., occur

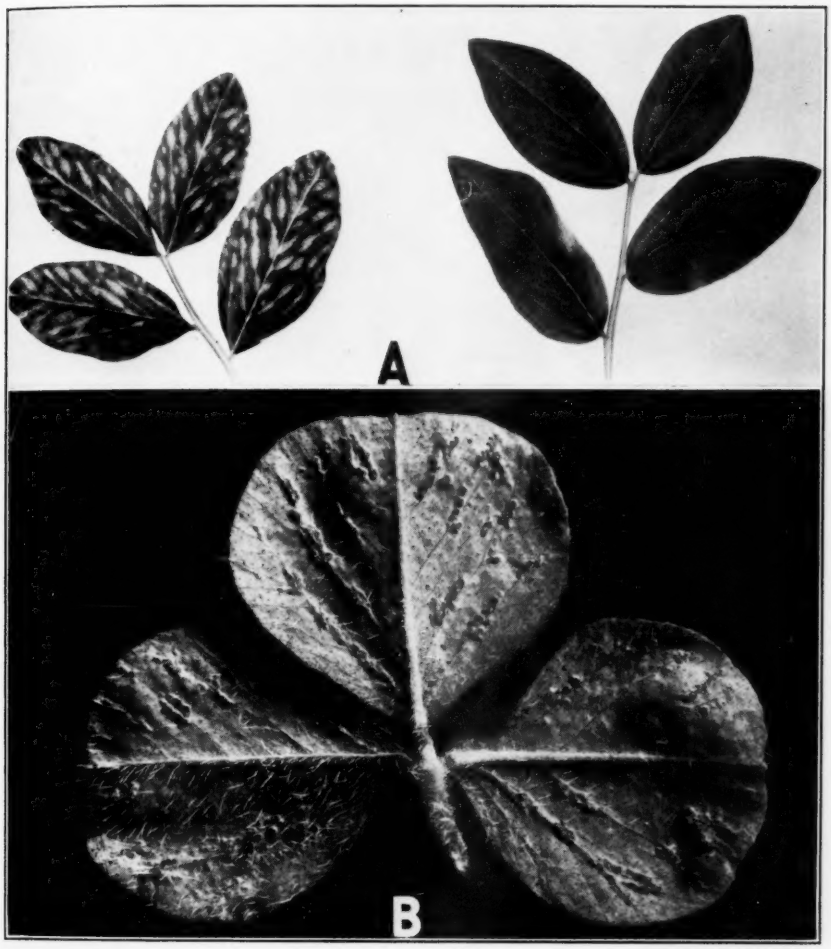

FIG. 1. A. *Vicia faba* leaves infected with pea mosaic from diseased *V. faba* by inoculation with the pea aphid. Left, from diseased plant; right, from healthy plant. B. Under surface of leaf from crimson clover plant showing enations produced by pea mosaic. Enlarged more than twice natural size.

on the under surface of the leaves. An advanced stage of the disease in Telephone pea is shown in figure 2, A.

In diseased sweet peas there is a pronounced wrinkling of the leaves. The mosaic pattern differs from that in the Telephone variety of garden pea in that the spots usually are elongated to such an extent that they might well be described as streaks. A diseased sweet-pea leaf is illustrated in figure 2, B.

Diseased plants of crimson clover are characterized by small yellowish

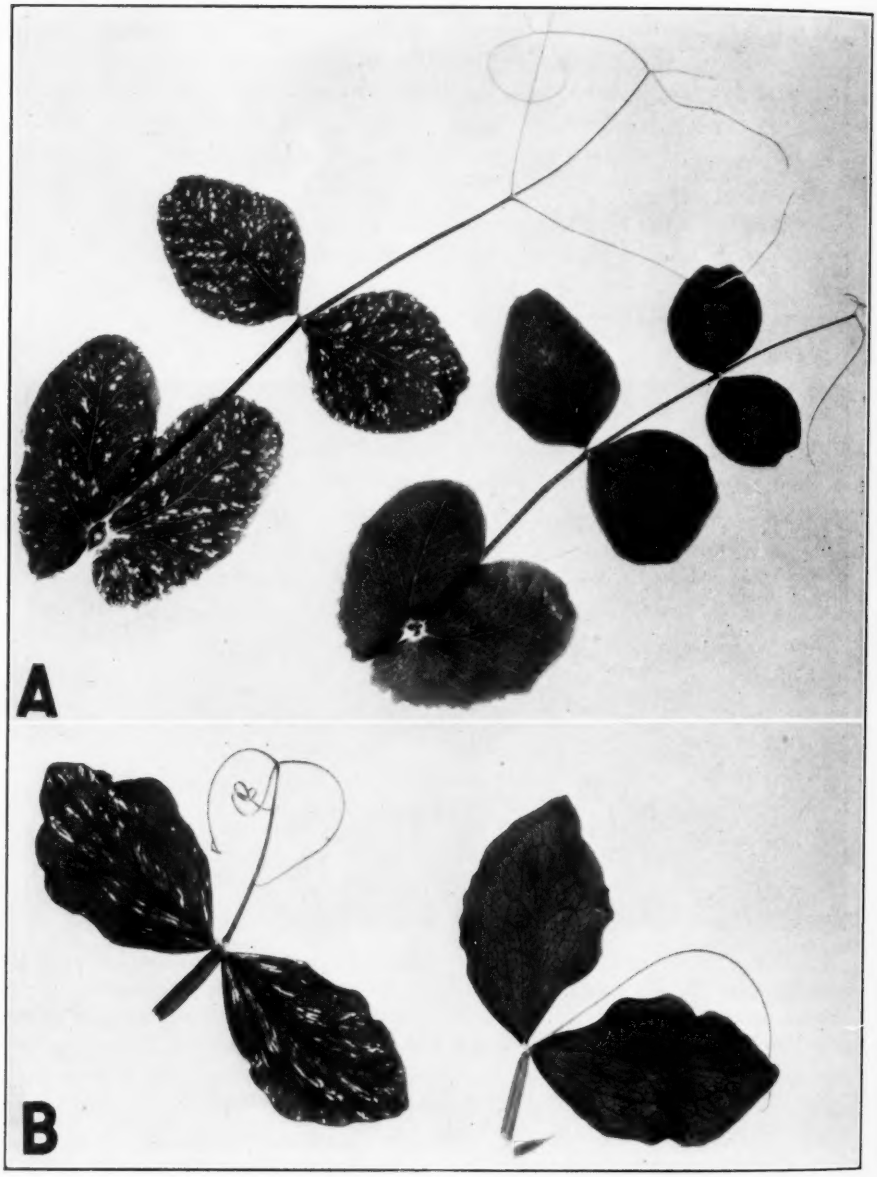

FIG. 2. A. Telephone pea infected with pea mosaic from diseased *Vicia faba* by inoculation with the pea aphid. B. Sweet pea infected with pea mosaic from diseased *Vicia faba* by inoculation with the pea aphid. In both A and B, leaves at left from diseased plant; those at right, from healthy plant.

spots on the upper surface of the leaves and distinct enations, such as are shown in figure 1, B, on the under surface of the leaves.

INCUBATION PERIOD OF THE VIRUS IN THE PEA APHID

A series of experiments was undertaken to determine whether or not transmission of the disease involves an incubation period of the virus in the insect. For this purpose colonies of the pea aphid were transferred from diseased to healthy *Vicia faba* plants. *Vicia faba* was used in all of the experiments for the reason that it is highly susceptible to pea mosaic, grows rapidly, serves as a satisfactory food for the aphids, and facilitates transfer of large colonies because of its broad smooth leaves. In preliminary experiments with colonies, it was found that if an incubation period existed it was less than 48 hours. Therefore, colonies of aphids were allowed to feed on diseased plants for periods of 2 to 12 hours, and were subsequently transferred to successions of healthy plants. The colonies consisted of from 50 to 200 insects in the nymphal stage except in one experiment where adults were used. The use of large numbers of aphids in colonies appeared to be desirable, since all of the insects placed on diseased plants do not acquire the virus even when allowed to feed for a 24-hour period. Moreover, single insects, even though carrying the virus, do not always infect healthy plants during a feeding period of 24 hours.

In one experiment conducted in a greenhouse, 5 colonies of pea aphids were fed for 8 hours on diseased plants and then transferred to a succession of healthy plants. The colonies in each case consisted of 50 adult insects. After removal from the diseased plants, 5 transfers were made at intervals of 4 hours and subsequent transfers at irregular intervals. The temperature was held at approximately 67° F. during the time the insects were on diseased plants and during the first 4 periods on healthy plants. For a short time during the 2nd day, due to bright sunlight, the temperature rose to 76° F. The infections obtained in this experiment are shown in table 1. Four of the colonies were infective. No infection, however, was obtained in any of the healthy plants exposed to the colonies during the first 4 periods. Two of the colonies became infective after an incubation period of not less than 16 nor more than 28 hours, and 2 of them after an incubation period of not less than 20 nor more than 36 hours. The experiment thus demonstrated an incubation period in adult pea aphids.

In a second experiment, similar to the preceding, nymphs were used instead of adults. Five colonies, consisting of 50 large nymphs each, were fed for 12 hours on diseased plants in a greenhouse that was held at a temperature that varied from 64° to 70° F. Twelve transfers were then made to healthy plants at 2-hour intervals, and subsequent transfers at irregular intervals. The infections resulting from these exposures showed that one

TABLE 1.—*Infections obtained with 5 colonies of adult pea aphids when fed for 8 hours on diseased Vicia faba plants and then transferred to a succession of healthy plants*

Period	Length of exposure	Approximate temperature during exposure	Succession of plants				
			Colony 1	Colony 2	Colony 3	Colony 4	Colony 5
1st	4 hrs.	67° F.	−	−	−	−	−
2nd	" "		−	−	−	−	−
3rd	" "		−	−	−	−	−
4th	" "		−	−	−	−	−
5th	" "	70° F.	+	+	−	−	−
6th	8 hrs.		+	+	+	+	−
7th	5 hrs.		+	+	−	+	−
8th	2 hrs.	76° F.	+	+	+	+	−
9th	17 hrs.		+	+	+	+	−
10th	24 hrs.		+	+	+	+	−
11th	" "		+	+	+	+	−
12th	" "		+	+	+	+	−

+ = Plant became infected.
− = Plant remained healthy.

colony became infective after an incubation period of not less than 10 nor more than 24 hours, 2 colonies became infective after an incubation period of not less than 20 nor more than 36 hours, and one colony was infective after a period of not less than 72 nor more than 96 hours. One colony failed to infect any plants on which it was placed. This experiment demonstrated an incubation period in nymphs of the pea aphid.

In one experiment conducted in a greenhouse, the temperature varied from 74° to 76° F. during the time the insects were on diseased plants, and was held above 70° F. during subsequent transfers. Five colonies, each consisting of 100 nymphs 2 to 4 days old, were allowed to feed for 6 hours on diseased plants and then transferred to a succession of healthy plants. Transfers were made at intervals of 2 hours to 9 sets of healthy plants and thereafter at irregular intervals. The infections resulting from the exposure of healthy plants to the colonies of aphids in this experiment are shown in table 2. Plants exposed to 2 of the colonies during the first period became infected. Two colonies, however, became infective only after an incubation period of not less than 6 nor more than 14 hours. For some unknown reason, one colony infected but a single plant. This experiment, therefore, demonstrated an incubation period in 2 colonies, but failed to demonstrate an incubation period in 2 other colonies, because some of the insects were infective within less than 8 hours after first feeding on diseased plants.

TABLE 2.—*Infections obtained with 5 colonies of pea aphids in the nymphal stage when fed for 6 hours on diseased plants and then transferred to a succession of healthy plants*

Period	Length of exposure	Approximate temperature during exposure	Succession of plants				
			Colony 1	Colony 2	Colony 3	Colony 4	Colony 5
1st	2 hrs.	72° F.	+	−	−	+	−
2nd	" "	"	−	−	−	+	−
3rd	" "	70–72° F.	+	−	−	+	−
4th	" "	"	+	+	+	+	−
5th	" "	"	+	−	−	+	−
6th	" "	"	+	+	+	+	−
7th	" "	"	+	+	+	+	−
8th	" "	70–80° F.	+	−	+	+	+
9th	" "	"	+	+	+	+	−
10th	24 hrs.		+	+	+	+	−
11th	" "		+	+	+	+	−
12th	" "		+	+	+	+	−

+ = Plant became infected.
− = Plant remained healthy.

An experiment was, therefore, conducted in which exposure to diseased plants was shortened. Five colonies were allowed to feed for 2 hours on mosaiced plants. The colonies consisted of 200 nymphs each. Transfers to healthy plants were made at intervals of 2 hours. The greenhouse was held at a temperature of approximately 80° F. while the insects were on diseased plants. Due to weather conditions, the temperature varied during the remainder of the experiment from 72° F. to 90° F. In making transfers of such large numbers of aphids at frequent intervals, some loss of individuals occurred. A count made at the 12th transfer showed that the number of insects remaining in the colonies varied from 165 to 190. The infections resulting from exposure of healthy plants to the colonies of aphids in this experiment are shown in table 3. All of the plants exposed to the colonies during the 1st and 2nd periods remained healthy. A plant exposed to one of the colonies during the 3rd period of the series became infected, as did a plant exposed to another colony in the 4th period. One colony became infective during the 7th period, and another during the 9th period. One infection obtained in this experiment showed that at high temperatures (80° F. in this case) some of the insects became infective within a period of not more than 8 hours after they first fed on diseased plants. Even with transfers of 200 insects to each plant, no infections occurred in plants exposed during the 1st and 2nd periods after removal from diseased plants. This showed that even at high temperatures the

TABLE 3.—*Infections obtained with 5 colonies of pea aphids in the nymphal stage when fed for 2 hours on diseased plants and then transferred to a succession of healthy plants*

Period	Length of exposure	Approximate temperature during exposure	Succession of plants				
			Colony 1	Colony 2	Colony 3	Colony 4	Colony 5
1st	2 hrs.	80° F.	−	−	−	−	−
2nd	" "	82° F.	−	−	−	−	−
3rd	" "	80° F.	−	−	−	−	+
4th	" "	90° F.	−	+	−	−	−
5th	" "		−	−	−	−	−
6th	" "	76° F.	−	−	−	−	−
7th	" "	72° F.	−	−	−	+	+
8th	" "		−	+	−	+	+
9th	" "	74° F.	−	+	+	+	+
10th	" "		−	+	−	+	+
11th	" "	82° F.	−	+	+	+	+
12th	24 hrs.		−	+	+	+	+
13th	" "		−	+	D	+	+

+ = Plant became infected.
− = Plant remained healthy.
D = Plant died.

insects do not become infective immediately after feeding on diseased plants. The incubation period varied from not less than 4 nor more than 8 hours to not less than 16 nor more than 20 hours in the different colonies.

One experiment was conducted in a laboratory where the temperature was maintained uniformly at 70° to 71° F. Two hundred and fifty nymphs were fed for 3 hours on diseased plants and then transferred in colonies, consisting of 50 insects each, to healthy plants at intervals of 3 hours. In this experiment all of the plants exposed to the colonies during the 1st, 2nd, and 3rd periods remained healthy. One plant of the set exposed during the 4th period of the series became infected; 2 plants exposed during the 5th period became infected; and 4 exposed during the 6th period became infected. All 5 of the plants exposed during the 7th period became infected. In the series of plants on which control colonies fed during the 6th period, one infection occurred. It is believed that this was due to accidental exposure to one or more infective aphids. The experiment again demonstrated an incubation period in the insects. At a uniform temperature of 70° to 71° F., the period was not less than 9 nor more than 24 hours.

One test was carried out in a dark cool cellar. The temperature of the cellar varied from 69° to 69.5° F. throughout the experiment. Five colonies, consisting of 100 nymphs each, were fed for 3 hours on diseased

Vicia faba plants and then transferred to a succession of healthy plants. The transfers to fresh plants were made at intervals of 3 hours. The infections resulting from exposure of healthy plants to the colonies in this experiment are shown in table 4. No infection occurred in any of the

TABLE 4.—*Infections obtained with 5 colonies of pea aphids in the nymphal stage when fed for 3 hours on diseased Vicia faba plants and then transferred to a succession of healthy plants under uniform conditions*

Period	Length of exposure	Approximate temperature during exposure	Succession of plants				
			Colony 1	Colony 2	Colony 3	Colony 4	Colony 5
1st	3 hrs.	69–69.5° F.	–	–	–	–	–
2nd	" "	" "	–	–	–	–	–
3rd	" "	" "	–	–	–	–	–
4th	" "	" "	–	–	+	–	–
5th	" "	" "	–	–	+	+	–
6th	" "	" "	–	–	+	+	–
7th	" "	" "	–	+	+	+	–
8th	24 hrs.	" "	+	+	+	+	–
9th	" "	" "	+	+	+	+	–

+ = Plant became infected.
– = Plant remained healthy.

plants exposed to the colonies during the 1st, 2nd, and 3rd periods. Colony 3 infected the plant on which it was placed during the 4th and each succeeding period of the series. Colony 4 infected the plant on which it was placed during the 5th and each succeeding period. Colony 2 infected the plant on which it was placed during the 7th and each succeeding period. Colony 1 became infective during the 8th period of the series. Colony 5 failed to infect any of the plants on which it was placed. This experiment again demonstrated an incubation period of the virus in the insects. The length of the incubation period as shown by infections varied even with colonies kept at a uniform temperature in the dark. At a temperature of 69° to 69.5° F., the incubation period varied from not less than 9 nor more than 15 hours to not less than 21 nor more than 48 hours in different colonies.

In the 6 experiments described above, approximately 2750 aphids in 30 colonies were fed for short periods on diseased plants and then transferred to several series of healthy plants. Of the 30 colonies of aphids, 4 failed to transmit the virus and one infected only a single plant. Two of the colonies infected the first plants on which they were placed after removal from diseased plants. These 2 colonies had fed for 6 hours on diseased plants in a greenhouse held at temperatures above 72° F. An incubation

period was definitely demonstrated for the remaining 23 colonies. In 20 of the colonies this period varied from not less than 9 nor more than 15 hours to not less than 21 nor more than 48 hours; in one colony a period of not less than 72 nor more than 96 hours was required; while in 2 colonies, held at temperatures of from 80° to 90° F., there was an incubation period of not less than 4 nor more than 10 hours.

RETENTION OF THE VIRUS BY THE PEA APHID

Two methods were used for the purpose of determining whether or not the virus is retained by the pea aphid for a considerable period of time. By the first method, pea aphids were fed on diseased plants and then transferred at frequent intervals to a succession of healthy susceptible plants. By the second method, pea aphids were fed on diseased plants and then transferred for a period of time to immune plants, after which they were transferred back to healthy susceptible plants.

Retention of the Virus by the Pea Aphid when fed on Susceptible Plants. *Vicia faba* was used in these experiments, since it had proved to be such a favorable plant on which to handle pea aphids. An objection to be considered, however, was the possibility that the insects might reacquire the virus from susceptible plants. Tests were, therefore, conducted in which it was found that freshly inoculated plants of *V. faba* did not serve as a source of virus for noninfective aphids for a period of at least 48 hours. From this it was concluded that by making transfers to fresh plants at daily or shorter intervals, the likelihood that the insects would reacquire the virus was remote.

In one experiment, 5 colonies of aphids in the nymphal stage were fed for 6 hours on diseased *Vicia faba* plants and then transferred to a succession of healthy plants. The nymphs were 1 to 3 days old when the experiment was started. After removal from diseased plants, 9 transfers to fresh plants were made at intervals of 2 hours, after which the insects were transferred to fresh plants every day. The colonies consisted of approximately 75 individuals each at the 10th transfer. During the 3rd week, the numbers began to dwindle and infections obtained in the 4th week were from a few surviving individuals. The results given by this test are shown in table 5. For some unknown reason, one of the colonies infected only 3 plants. This colony differed from the other colonies only in regard to the diseased plant upon which it had fed. Four of the colonies infected fresh plants each day for 17 days, and one of the 4 infected each of 9 plants on which it fed during the first day and continued to infect plants every day for 29 days. The experiment was discontinued after the 31st day, as only a few weakened individuals remained alive. These results demonstrated that pea aphids, when fed on diseased plants as small nymphs, acquired and retained the virus for periods up to 29 days.

TABLE 5.—*Inoculations with 5 colonies of pea aphids fed, when small nymphs, for 6 hours on diseased Vicia faba and then transferred to a succession of healthy plants, during the life of the insects*

Period	Length of exposure	Successions of plants				
		Colony 1	Colony 2	Colony 3	Colony 4	Colony 5
9 periods	2 hrs. each	8+	3+	5+	9+	1+
2nd day	24 hrs.	+	+	+	+	−
3rd day	" "	+	+	+	+	−
4th day	" "	+	+	+	+	−
5th day	" "	+	+	+	+	−
6th day	" "	+	+	+	+	−
7th day	" "	+	+	+	+	−
8th day	" "	+	+	+	+	−
9th day	" "	+	+	+	+	−
10th day	" "	+	+	+	+	−
11th day	" "	+	+	+	+	−
12th day	" "	+	+	+	+	−
13th day	" "	+	+	+	+	−
14th day	" "	+	+	+	+	−
15th day	" "	+	+	+	+	−
16th day	" "	+	+	+	+	−
17th day	" "	+	+	+	+	−
18th day	" "	+	+	+	+	−
19th day	" "	+	−	−	+	+
20th day	" "	+	+	−	+	−
21st day	" "	−	+	−	+	−
22nd day	" "	+	+	−	+	−
23rd day	" "	−	−	−	+	−
24th day	" "	−	+	−	+	−
25th day	" "	−	−	−	+	−
26th day	" "	−	−	−	+	−
27th day	" "	−	−	−	+	+
28th day	" "	−	−		+	−
29th day	" "	−	−		+	−
30th day	" "				−	−
31st day	" "				−	−

+ = Plant became infected.
− = Plant remained healthy.

To determine the effectiveness of single insects in retaining the virus, a record was made of the infections obtained from 25 individual pea aphids when fed for 1 or 2 days on diseased *Vicia faba* plants and then transferred every day to healthy plants. The record is shown in table 6.

Considerable variation was observed in the ability of different aphids to infect plants. Some of the aphids infected plants consistently on consecutive days. Others failed on alternate days, or for several days and

TABLE 6.—*Infections obtained with individuals of the pea aphid when fed on diseased Vicia faba plan and then transferred to a succession of healthy plants*

Period	Nymphs on diseased plant 24 hrs. 5/10–11/33					Alate adults on diseased plant 24 hrs. 7/8–9/33					Apterous adults on diseased plant 24 hrs. 7/8–9/33					Nymphs on diseased plant 48 hrs. 8/8–10/33					Nymphs on diseased plant 48 hrs. 8/8–10/33				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1st day	+	−	−	+	+	−	+	+	−	−	+	+	−	−	−	+	−	−	−	−	+	+	+	+	+
2nd day	+	+	+	+	−	+	+	+	+	+	+	+	+	−	−	+	−	−	−	−	+	+	−	+	+
3rd day	+	+	+	+	+	+	+	+	+	*	+	+	+	−	−	+	−	−	−	−	+	+	+	−	−
4th day	+	+	+	+	−	−	+	+	+	+	+	+	−	−	−	+	−	−	−	−	+	+	+	+	+
5th day	+	+	−	+	+	+	+	+	+	+	+	+	−	−	−	+	−	−	−	−	+	−	+	+	+
6th day	−	−	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	+	+	+	+	+
7th day	−	+	+	+	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	+	−	+	−	−
8th day	D	+	+	+	−	+	+	+	+	+	+	+	+	−	−	+	−	−	−	−	+	+	−	+	+
9th day		+	+	−	+	+	+	+	+	+	+	+	+	−	−	+	−	−	−	−	−	−	+	+	+
10th day		+	−	−	−	+	+	+	−	−	−	−	−	−	−	+	−	−	−	−	+	L	−	−	−
11th day		−	−	+	+	−	−	+	+	−	−	−	−	−	−	+	−	−	−	−	+		+	+	+
12th day		+	+	−	+	−	−	+	+	+	−	+	−	−	−	−	−	−	−	−	+		−	−	−
13th day		−	−	−	−	−	+	+	+	+	A	A	A	A	A	+	−	−	−	−	+		−	−	−
14th day		+	−	−	−	−	+	−	−	−						+	−	−	−	−	+		L	+	+
15th day		−	D	−	−	−	+	+	+	+						+	−	−	−	−	+			+	+
16th day		+		−	−	A	A	A	A	A						−	−	−	−	−	−			−	−
17th day		−		−	−											A	A	A	A	A	A			L	
18th day		−		D	−																				
19th day		−			−																				
20th day		+			−																				
21st day		D			−																				
22nd day					−																				
23rd day					+																				
24th day					−																				
25th day					−																				
26th day					D																				

+ = Plant became infected.
− = Plant remained healthy.
* = Plant dead from fumigation.
D = Insect dead.
L = Insect lost.
A = Discontinued with insects still alive.

then again infected plants. The longest observed time in which a single aphid remained infective was 23 days. This result was obtained with an insect that was fed on a diseased plant when 3 days old and that infected a plant on which it was placed 23 days later.

The most consistent performance was observed in 5 individuals that were winged adults at the time they were placed on diseased plants. In a period of 15 days, these 5 aphids infected 57 of the 75 plants on which they were placed. Four of the 5 infected plants on the 15th day. One infected 14 of the 15 plants on which it was placed, failing only on the 14th day. These insects had completed reproductive activity and appeared sluggish and weakened during the last few days. They were, therefore, not carried beyond the 15th day.

It was found that some of the insects, after being infective for a time, failed to infect plants in later periods, while others were infective until within a day or two of death. The tests did not show whether this failure was due to a loss of the virus by the insects or to some factor, such as a weakened condition, that might affect the feeding habits of the insects. These tests confirmed the results obtained in experiments with colonies. They also demonstrated the effectiveness of single insects as carriers of the disease. The retention of the virus by single insects was shown for periods up to 23 days.

Retention of the Virus by the Pea Aphid when fed on Immune Plants. It was believed that the possibility of aphids reacquiring the virus from plants on which they were allowed to feed would be reduced to a minimum if immune plants were used. Information, however, was meager concerning plants that were at the same time immune from the virus and satisfactory as food for the aphids. Among the plants that had failed to become infected after inoculation with this virus by the pea aphid was the Spanish peanut. It appeared also to be satisfactory as a food plant for this aphid. The Spanish peanut was, therefore, used in one experiment on retention of the virus by the pea aphid. Two hundred and fifty nymphs were placed on diseased *Vicia faba* plants and allowed to feed for 2 days. They were then placed for one day on 10 healthy *V. faba* plants before transfer to peanuts. They remained on the peanut plants for 7 days, being disturbed only once in order to remove accumulating progeny. When removed from the peanuts, they were transferred successively to 2 lots of 10 plants each of *V. faba*. No evidence of infection became apparent in any of the peanut plants, though they were kept under observation in a greenhouse for 2 months. All of the *V. faba* plants in the 2 lots became infected, demonstrating that the pea aphids had retained the virus for 7 days when fed on an immune plant. Experiments have not as yet been carried out that would show retention of the virus by the pea aphid when fed for more extended periods on immune plants.

TRANSMISSION BY OTHER INSECTS THAN THE PEA APHID

In order to determine whether other insects than the pea aphid can transmit pea mosaic, tests were made with the bean aphid, *Aphia rumicis* L., and the potato aphid, *Macrosiphum gei* (Koch).

The bean aphid was observed in abundance at times on *Vicia faba* in the field, and the possibility was suggested that it might carry the disease. Colonies of the bean aphid were maintained continuously on *V. faba* plants in a greenhouse. For the purpose of determining whether or not they would transmit the disease, bean aphids that had developed on diseased *V. faba* were transferred to small healthy plants. From 25 to several hundred insects were transferred to each plant and allowed to feed for at least 2 days. One lot of 20 plants was placed in a screened cage for one week beside diseased plants on which aphids were feeding, to permit of natural migration from diseased to healthy plants. In all, 100 plants of *V. faba* were exposed to bean aphids that had developed on diseased plants. Since no infection appeared in any of these plants, it is concluded that the bean aphid is not able to transmit the disease.

Tests with the potato aphid as a possible vector of the mosaic were undertaken, because it is closely related to the pea aphid and is known to feed at times on peas and clovers. In these tests the pink asexual form of the potato aphid was used rather than the green form, in order that it might be readily distinguished from the pea aphid. Colonies were maintained on tomato plants in screened cages.

In one test potato aphids were fed for 2 days on diseased *Vicia faba* plants and then transferred to 20 healthy plants. They remained on the healthy plants one day and were then transferred to a second lot of 20 healthy plants and allowed to feed for one day. Twenty to 30 aphids were placed on each healthy plant. Of the 20 plants exposed the first day, 19 became infected, and of the 20 plants exposed the 2nd day, 19 again became infected. The experiment demonstrated that the potato aphid, as well as the pea aphid, is capable of transmitting the disease. No attempt has been made to determine whether or not the pea-mosaic virus requires an incubation period in the potato aphid before it is transmitted by this vector.

Experiments to determine whether or not other insects are capable of transmitting pea mosaic have not been undertaken. The studies reported above, however, definitely establish the fact that the disease is transmitted by both the pea aphid and the potato aphid, and that it is not transmitted by the bean aphid. It is concluded, therefore, that, although the disease can be transmitted by more than one species of insect, the ability to transmit is nevertheless restricted to certain species.

TRANSMISSION BY MECHANICAL METHODS

To determine if pea mosaic can be transmitted by mechanical methods, as well as by the pea aphid and potato aphid, a total of 772 *Vicia faba* plants and 150 garden pea plants were inoculated with juice expressed from diseased *V. faba* plants. Eighty garden pea and 20 *V. faba* plants were inoculated with juice expressed from diseased garden peas. These were grown beside control plants that remained healthy. Only 3 of the inoculated plants became infected. Of the 3 that became infected, one was in a lot of 40 plants inoculated by punctures with 000 insect pins moistened with juice from diseased *V. faba* plants. Since infection did not appear until 21 days after inoculation, it was felt that contamination might have occurred. Two of the plants that became infected were in a lot of 100 *V. faba* plants inoculated with expressed juice from diseased *V. faba* plants to which norit was added at the rate of 1 part in 20. The juice was rubbed into the surface of the leaves with bandage gauze. Infection appeared 13 days after inoculation. The number of infections obtained from these inoculations was so small as to be unsatisfactory for drawing a final conclusion as to transmissibility by mechanical means. They did demonstrate that the transmission of this mosaic by the usual mechanical methods is extremely difficult.

DISCUSSION

The experiments presented in this paper have shown that the pea aphid is not able to infect healthy plants immediately after first feeding on diseased plants, but that an interval, referred to as an incubation period of the virus in the insect, must elapse. However, once the insect acquires the virus, it is retained for considerable periods of time. These results indicate that the transmission of this disease by the pea aphid is similar as regards incubation period and retention to the transmission of curly top of beet, aster yellows, and streak of maize by leaf hoppers.

The nature of the incubation period and means by which the virus is retained in the insect are not known. It is possible that the virus may multiply or pass through a stage of development in the insects. On the other hand, the incubation period may merely represent the time required for the virus to diffuse through the body fluids or tissues to reach a point where it may be injected into the plant by the feeding of the insects.

Whatever the explanation, it is of interest that this mode of transmission appears to differ from that shown to occur in the transmission of cucumber mosaic and yellow dwarf of onions by aphids, in which transmission is without an interval or incubation period and in which retention of the ability to infect by the insects is for only a brief period.

Curly top of beet, aster yellows, and streak of maize are known to be

carried only through the agency of single species of leaf hopper. It is of interest that in the case of pea mosaic, in which a similar mode of transmission has been found, 2 species of aphids, the pea aphid and the potato aphid, have been shown to be carriers. The ability to transmit the disease is nevertheless restricted to certain species. The bean aphid is not able to spread the disease. It is also of interest that pea mosaic is difficult to transmit by mechanical methods.

SUMMARY

This paper presents the results of experiments that demonstrate an incubation period of pea mosaic in the aphid, *Macrosiphum pisi* (Kaltenbach).

By exposing successions of healthy *Vicia faba* plants to colonies of pea aphids that were allowed to feed for short periods on diseased plants, an incubation period of the virus in the aphids was shown to exist. This was definitely demonstrated for 23 colonies. In 20 of the colonies the period varied from not less than 9 nor more than 15 hours to not less than 21 nor more than 48 hours; in one colony a period of not less than 72 nor more than 96 hours was required; while in 2 colonies, held at temperatures of from 80° to 90° F., there was an incubation period of not less than 4 nor more than 10 hours.

Pea aphids that acquire the virus after feeding on diseased plants retain it for considerable periods of time. Retention of the virus was demonstrated with single insects, as well as with colonies. One colony retained the virus over a period of 29 days.

The potato aphid, as well as the pea aphid, transmits the disease, but the bean aphid does not.

It was found difficult to transmit this mosaic by mechanical methods.

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EFFECT OF NITROGEN SUPPLY ON HOST SUSCEPTIBILITY TO VIRUS INFECTION[1]

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It has long been known from studies dealing with host susceptibility to parasitic infection that the environmental factors, mineral nutrition, light, temperature, and humidity, influence the host response to infection. In the field of plant-virus investigations several of these factors have been studied, but a review of the literature shows that very little consideration has been given to the possible effects of mineral nutrition on host susceptibility to virus infection. The object of this paper is to present the results of experiments dealing with the influence of variations in the nitrogen supply on the susceptibility of 3 different hosts to tobacco mosaic.

REVIEW OF LITERATURE

The influence of plant succulence in altering host tolerance for virus diseases has been recognized since 1922. Observations by Dickson (4) on tobacco mosaic, Elmer (6) on tomato mosaic, Kraybill and Eckerson (12) on tomato mosaic, Kirkpatrick (11) on leaf curl of cotton, Drake and coworkers (5) on yellow dwarf of onion, and Carter (3) on yellow spot of pineapple indicate that there is a definite relationship between host succulence and susceptibility to infection. Samuel and Bald (17) found that dormant seedlings of *Nicotiana glutinosa* L. were considerably more resistant to infection with tobacco-mosaic virus than vegetative seedlings.

Little work has been recorded on the factors underlying host succulence. The few brief reports so far published on nitrogen nutrition and host susceptibility to virus infection may be summarized briefly as follows: Schultz and Folsom (18) observed that during the first part of the season the percentage of mosaic on potatoes was lowered to some extent by high nitrogen and high potassium fertilizers. On the other hand, when healthy potato plants were interspersed with mosaicked plants, Janssen (9) found that the lowest incidence of mosaic was almost uniformly among the plants receiving little or no nitrogen. In a similar test with leaf roll of potato, he found that the resistance of plants in low-nitrogen plots was even more conspicuous. Quanjer (14) concluded from his investigations and those of his collaborators that pseudo-net-necrosis spreads more readily to healthy plants when the nitrogen supply is high. Ainsworth (1) observed on permanent soil plots receiving the same fertilizer treatment each year that the

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number of tomato plants affected by tomato stripe on a plot receiving complete fertilizer was 5.0 per cent, but when nitrogen was omitted from the complete fertilizer it was only 1.6 per cent. Samuel and Bald (17) found that occasional watering with ammonium phosphate considerably increased the susceptibility of *Nicotiana glutinosa* to tobacco-mosaic virus.

The only detailed experiment published to date on the effect of nutrition on host susceptibility to virus diseases is that of Volk (20) who grew tomato and tobacco plants in nutrient sand cultures. The tomato plants were inoculated with spot-mosaic virus (Fleckmosaik) when they were 8 weeks old. The tobacco was inoculated at the pre-flower stage by injecting expressed sap from a mosaic-diseased tobacco plant under the epidermis of the lowermost leaf. His results, with respect to nitrogen nutrition, show that the percentage of diseased plants is increased with increasing amounts of nitrogen. The effect of nitrogen on growth was not considered.

MATERIALS AND METHODS

The experiments reported in this paper were planned to study the effect of nitrogen supply on growth and host susceptibility to tobacco mosaic. The host plants were *Nicotiana glutinosa* L., *N. tabacum* L. var. Turkish, and *Phaseolus vulgaris* L. var. Early Golden Cluster. Growth was measured by recording the green weight and height of representative plants in each test at time of inoculation. Since the resultant graphs of these two growth criteria proved to be similar, only the green weight data are here presented. Susceptibility of plants receiving different amounts of nitrogen was determined by counting the number of lesions per 100 pin punctures on tobacco and *N. glutinosa* and by the number of lesions per leaf on bean plants.

Ordinary green tobacco-mosaic virus, designated by Johnson (10) as tobacco virus 1, was used to infect *Nicotiana glutinosa* and bean as it forms necrotic primary lesions on both of these hosts. The bean variety used, Early Golden Cluster, was shown by Price (13) to produce large numbers of primary lesions when inoculated with green tobacco-mosaic virus. Since much is already known concerning the reaction of Turkish tobacco to tobacco mosaic, this variety was chosen as the third host, even though the chlorotic primary lesions of tobacco mosaic on tobacco are often obscure and have to be stained with potassium iodide-iodine solution (8) to make them sufficiently distinct to be counted. On tobacco a yellow tobacco-mosaic virus, Johnson's tobacco virus 6, was used in preference to the ordinary green tobacco-mosaic virus previously mentioned because of its more drastic action on chloroplasts. Ordinary green tobacco mosaic (8) exerts only a slight inhibitory action on starch formation, whereas yellow tobacco mosaic (16) inhibits starch formation completely. For this reason, lesions of yellow tobacco mosaic are easier to demonstrate by the iodine method. In

order to record the number of lesions on tobacco, the inoculated leaves were removed from the plants late in the afternoon and placed in 95 per cent ethyl alcohol to kill the cells and to dissolve out the chlorophyll. The leaves were then stained in a weak aqueous solution of iodine in potassium iodide. Primary lesions appeared as small yellow spots against a dark background of healthy tissue. The two strains of tobacco-mosaic virus, green and yellow, were obtained several years ago from James Johnson and since that time have been passed through tobacco plants many times before being used in the experiments here reported.

Bean seedlings were inoculated by lightly rubbing the entire upper surface of the leaves with a small cheesecloth pad saturated with a 1–100 dilution of freshly extracted tobacco-mosaic virus. The juice was prepared by grinding several leaves of a diseased tobacco plant in a sterile mortar and then diluting with water. Tobacco and *Nicotiana glutinosa* were inoculated by the pin-puncture method, as described by Holmes (7). A set of 20 small black enamel insect pins, size No. 000, were bound between pot labels that served as a handle. With such a set, it was possible to make 20 pricks by each thrust. If the punctures were made interveinally, very little pressure was needed to force the pins through the leaf. The pins were dipped in freshly extracted undiluted juice from a diseased plant and then pricked into the test plant.

Beans were planted, 3 in each 4-inch pot, in washed, white quartz sand. Seeds of tobacco and *Nicotiana glutinosa* were germinated in sand and the seedlings kept moist with a complete nutrient solution. When the seedlings had 3 or 4 small leaves and were about 2 cm. in height, they were potted in 4-inch pots in a soil that was very low in fertility. Although sand would have been preferable to soil for the Nicotiana species, the latter was used because of the time required to feed sand cultures daily for 5 weeks. Since beans were usually on nutrient treatment for only 8 days, they could be cultured in sand without involving an undue amount of labor.

The nutrient culture technique was similar with all three hosts. The plants were fed 100 cc. per pot with a modified Shive's (19) 3-salt solution as a basic nutrient. To this solution was added the calculated amount of nitrogen as $Ca(NO_3)_2$ for each treatment. Ammonium nitrate was not used as a source of nitrogen because both tobacco and bean are somewhat sensitive to ammonium nitrogen. Ammonium nitrogen also masks to some extent the primary lesions on tobacco, as shown by the iodine staining technique. The composition of the basic solution, which was well balanced with regard to all the essential elements except nitrogen, is given in table 1. The tobacco and *Nicotiana glutinosa* plants in soil were fed twice each week; the beans in sand were fed daily.

Two or more tests extending over several seasons of the year were carried out with each host. During the experimental period the tempera-

TABLE 1.—*Composition of basic minus-nitrogen nutrient solution*

Salts used	Molecular proportions	Partial volume-molecular concentrations[a]
KH_2PO_4	3	.0036
$CaCl_2$	2	.0024
$MgSO_4$	3	.0036

[a] Total osmotic concentration value approximately 0.5 atmosphere.

ture of the greenhouses was held between 70° and 75° F. at night and between 70° and 80° F. during the day. Although no aphids or other insects were noticed, the greenhouses were fumigated weekly with nicotine to avoid any possible contamination by insect transmission. In all tests the pots were set in saucers and spaced to avoid crowding of foliage. For clearness, the results obtained with each host will be presented separately.

INFLUENCE OF NITROGEN ON SUSCEPTIBILITY OF NICOTIANA GLUTINOSA TO GREEN TOBACCO MOSAIC

The plants were inoculated when the blossom buds first began to show. At this stage of growth the number of leaves per plant suitable for inoculation ranged from 4 in the slow-growing plants to 10 in the most vegetative ones. One hundred pin punctures were made interveinally in each leaf. The final record of the number of necrotic primary lesions visible was made on the 5th day after inoculation.

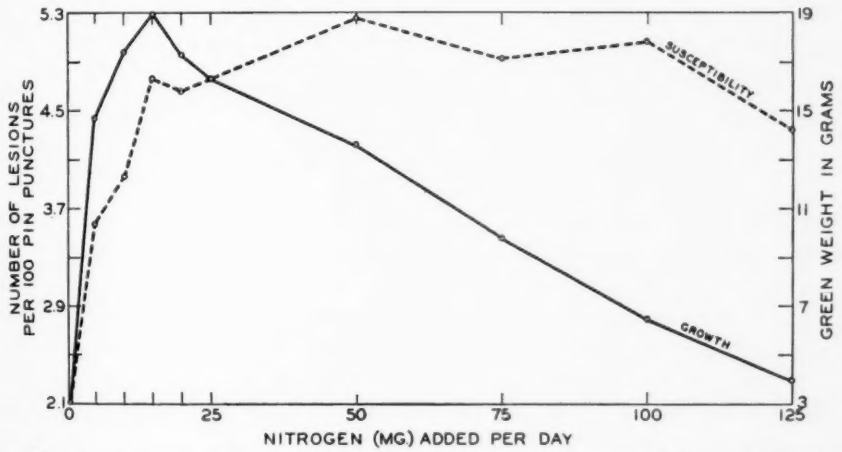

FIG. 1. Influence of nitrogen supply on growth and susceptibility of *Nicotiana glutinosa* to green tobacco mosaic. The solid line shows the average green weight of plants at time of inoculation; the broken line, the number of lesions per 100 pin punctures.

The average results of two tests with *Nicotiana glutinosa* are shown graphically in figure 1. The solid line represents the average green weight at time of inoculation of the plants receiving the amounts of nitrogen indicated along the base line. The broken line represents the susceptibility of similar plants to green tobacco mosaic. From these graphs it is evident that growth is influenced very markedly by nitrogen. Maximum growth occurs at a relatively low nitrogen level (15 mg. per day) and rapidly falls off as nitrogen is increased. High susceptibility, on the other hand, occurs over a wide range of nitrogen levels (15–100 mg. nitrogen per day). Thus, susceptibility is not definitely correlated with rapidity of growth.

INFLUENCE OF NITROGEN ON SUSCEPTIBILITY OF BEAN TO GREEN TOBACCO MOSAIC

As soon as the plumules became visible between the cotyledons, usually 6 or 7 days after planting, the nutrient treatments were started. About 6 days later, when the first true leaves were fully developed, the plants were trimmed by removing the growing tip of each to prevent intertwining during subsequent growth, and then inoculated. Small necrotic lesions appeared on the leaves the 2nd or 3rd day after inoculation. The lesions were usually counted on the 4th or 5th day after inoculation when even the smallest were large enough to be seen easily.

On plants receiving low amounts of nitrogen, the lesions appeared as reddish brown spots 1 to 2 mm. in diameter, with grayish brown necrotic centers. With high nitrogen nutrition the lesions were considerably smaller, ranged in color from dark reddish brown to almost black, and had no light-colored centers. The lesions appeared earlier at low and medium nitrogen levels than at high levels.

The bean plant makes such rapid growth that marked response to nutritive treatment, both in growth and susceptibility, is observed after a few days of nutrient treatment. The results obtained in 4 tests on the effect of nitrogen nutrition on the susceptibility of bean to tobacco mosaic are presented in table 2. It is readily seen from this table that the susceptibility of the bean is definitely influenced by variation in nitrogen supply. Maximum growth, as measured by green weight, was produced by moderately low amounts of nitrogen, between 20 and 60 mg. per day. Figure 2 shows typical plants at several different nitrogen levels at time of inoculation. Susceptibility did not reach its maximum until the nitrogen level was raised to 80 or 100 mg. per day. This relationship between growth and susceptibility as shown by the bean at various nitrogen levels is expressed graphically in figure 3. Data for these graphs were taken from table 2. The solid line represents the average green weight, in grams, of representative plants at time of inoculation. The broken line represents the average

TABLE 2.—*Response shown by bean to various nitrogen treatments and the effect of such treatments on the host susceptibility to green tobacco mosaic*

Nitrogen added per day (mg.)	Green weight (gm.) at time of inoculation				Number of lesions per leaf				
	Test No. 2	Test No. 3	Test No. 4	Average	Test No. 1	Test No. 2	Test No. 3	Test No. 4	Average
0	2.4[a]	2.3[a]	2.1[a]	2.27	1[b]	1[b]	9[b]	15[b]	7
10					2	7	9	41	15
20	2.9	2.6	2.7	2.73	7	14	8	39	17
40	2.9	2.5	2.5	2.63	11	25	11	72	30
60	3.0	2.5	2.5	2.67	17	50	12	99	45
80	2.6	2.3	2.5	2.47	26	53	13	123	54
100	2.4	2.4	2.2	2.33	28	42	29	101	50
125					39	30	17	82	42
150	2.2	2.1	2.1	2.13	28	20	12	60	30
175					30	23	18	72	36
200	2.2	2.1	1.8	2.03	13	19	15	41	22
225						15	13	33	20
250		2.1	1.8	1.95		13	6	24	14
300	1.7	1.7	1.5	1.63		11	1	18	10

[a] Each value represents the average green weight of 32–43 plants.
[b] Each value represents the average number of lesions per leaf of 81–119 plants.

FIG. 2. Typical bean plants that have received 0, 20, 80, 100, 200, and 300 mg. of nitrogen per day, respectively, from left to right, photographed at time of inoculation. Note that after the 2nd pot from the left, the plants become smaller as the nitrogen level is raised. The plants in the 2 middle pots proved to be most susceptible.

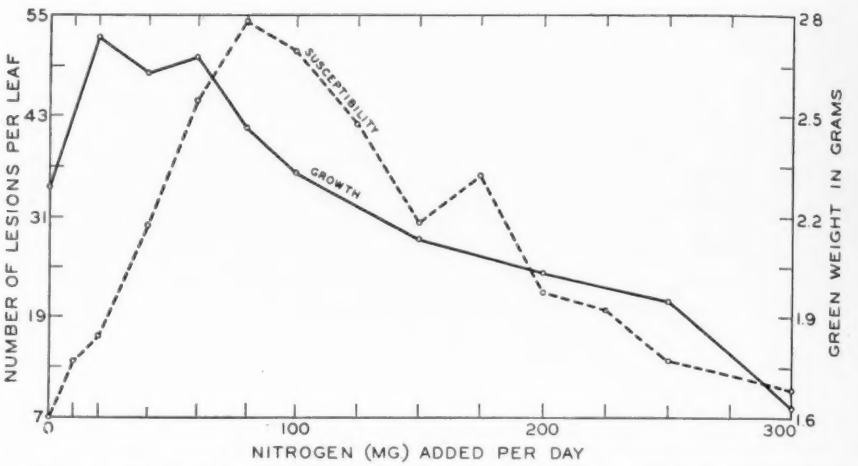

FIG. 3. Effect of nitrogen on growth and susceptibility of bean to green tobacco mosaic. The solid line shows the average green weight of plants at time of inoculation; the broken line, the average number of lesions per leaf when nitrogen is added as indicated along the base line.

number of primary lesions per leaf when the plants were supplied with the amounts of nitrogen indicated along the base line.

INFLUENCE OF NITROGEN ON SUSCEPTIBILITY OF TOBACCO TO YELLOW TOBACCO MOSAIC

After approximately 4 weeks of nutrient treatment when the tobaccos had reached the 12- or 13-leaf stage of development, representative plants from each treatment were inoculated with yellow tobacco-mosaic virus by pin punctures. The number of punctures per leaf ranged from 60 in the youngest leaf (about 4 cm. long) to 280 or 320 in the oldest leaf, depending on its size. On the 5th day after inoculation, before the primary lesions had fused or systemic symptoms had appeared, the youngest leaves were removed and treated by the iodine method as modified by Samuel (16) for yellow tobacco mosaic. Since the expression of primary symptoms is delayed in old tissue, the remaining leaves were not removed until the 7th day after inoculation.

Results of 3 tests on the effect of nitrogen on growth are recorded in table 3. From these data it is readily apparent that maximum growth, as measured by green weight, was produced when very small amounts of nitrogen, between 10 and 15 mg. per plant per day, were added. Figure 4 shows typical tobacco plants that have received 0, 10, 20, 75, and 125 mg. of nitrogen per day, respectively, photographed at time of inoculation. With large applications of nitrogen, growth was greatly retarded. When more than

FIG. 4. Typical tobacco plants that have received 0, 10, 20, 75, and 125 mg. of nitrogen per day, respectively, from left to right, photographed at time of inoculation. The 2 plants on the right were the most susceptible.

125 mg. of nitrogen per day were added, most of the plants died. Plants receiving large quantities of nitrogen were much darker in color than those that made the fastest growth. Plants that received no nitrogen made little growth. The leaves were stiff, leathery in texture, and yellowish in color. Several of the lowermost leaves died and dried up. In no other treatment did the plants show any evidence of nitrogen starvation.

TABLE 3.—*Effect of nitrogen on growth and susceptibility of tobacco to yellow tobacco mosaic*

Nitrogen added per day (mg.)	Green weight (gm.) at time of inoculation				Number of lesions per 100 pin punctures			
	Test No. 1	Test No. 2	Test No. 3	Average	Test No. 1	Test No. 2	Test No. 3	Average
0	11.0[a]	5.6[a]	2.0[a]	6.2	2.0[a]	1.9[a]	2.5[a]	2.1
5	27.0	23.0	27.5	25.8	4.6	2.4	4.5	3.8
10	38.4	31.0	31.2	33.5	4.7	2.9	4.7	4.1
15	38.4	30.8	33.0	34.1	4.3	3.6	4.0	4.0
20	34.2	27.6	29.4	30.4	4.6	5.0	4.3	4.6
25	33.9	26.0	28.8	29.6	5.5	4.2	4.5	4.7
50	23.9	17.4	18.2	19.8	5.5	5.0	5.4	5.3
75	13.4	11.2	10.4	11.7	5.7	5.8	7.3	6.3
100	6.6	5.2	5.8	5.9	3.5	7.7	7.4	6.2
125	2.6	3.8	3.7	3.4	3.5	4.4	7.6	5.2

[a] Average of 5 plants.

Table 3 also shows results obtained by inoculating sets of tobacco plants, similar to those harvested for green weight determinations, with yellow tobacco-mosaic virus. It is seen that susceptibility to virus infection gradually rises with increasing amounts of nitrogen up to a definite level (75–100 mg. nitrogen per day), beyond which susceptibility falls off gradually. No appreciable difference could be noted in the appearance of the primary lesions with any of the various nitrogen treatments.

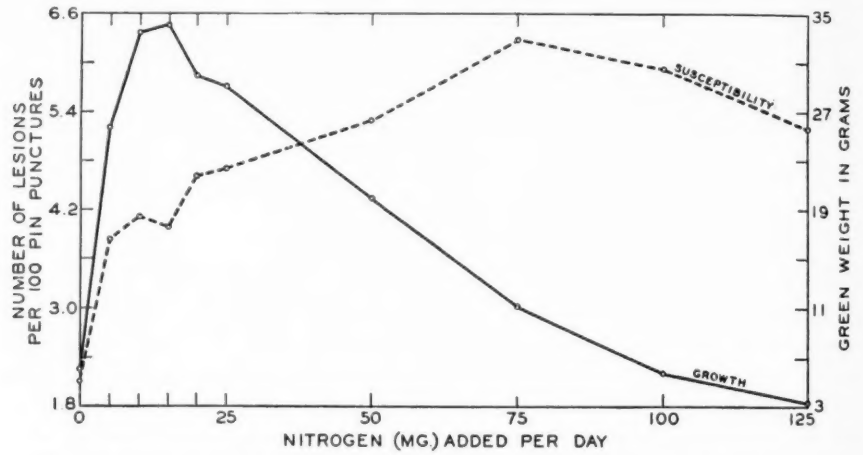

FIG. 5. Influence of nitrogen supply on growth and susceptibility of tobacco to infection with yellow tobacco mosaic. The solid line shows the average green weight of plants at time of inoculation; the broken line, the number of lesions per 100 pin punctures when nitrogen is added as indicated along the base line.

The relationship between growth and host susceptibility of tobacco as influenced by nitrogen nutrition is expressed graphically in figure 5. The solid line represents the average green weight at time of inoculation of plants supplied with nitrogen at the various levels. The broken line represents the susceptibility of similar plants to yellow tobacco mosaic. These graphs show that, with tobacco, highest susceptibility does not occur at nitrogen levels favoring the most rapid growth; in fact, the nitrogen levels producing maximum susceptibility exert a retarding influence on growth.

The question arises as to whether the response in growth and susceptibility was due to nitrogen or to calcium, since nitrogen was added as $Ca(NO_3)_2$ and calcium increased simultaneously with nitrogen. In order to answer this question, another test was carried out in which nitrogen only was varied by using NH_4NO_3 instead of $Ca(NO_3)_2$ as a source of nitrogen, and calcium was supplied as $CaCl_2$. The symptoms of ammonium toxicity were not severe enough to interfere materially with the lesion count at the end of the experiment. The results of this test are expressed graphically

in figure 6. The solid line shows the average green weight at time of inoculation; the broken line, the susceptibility of representative plants when in-

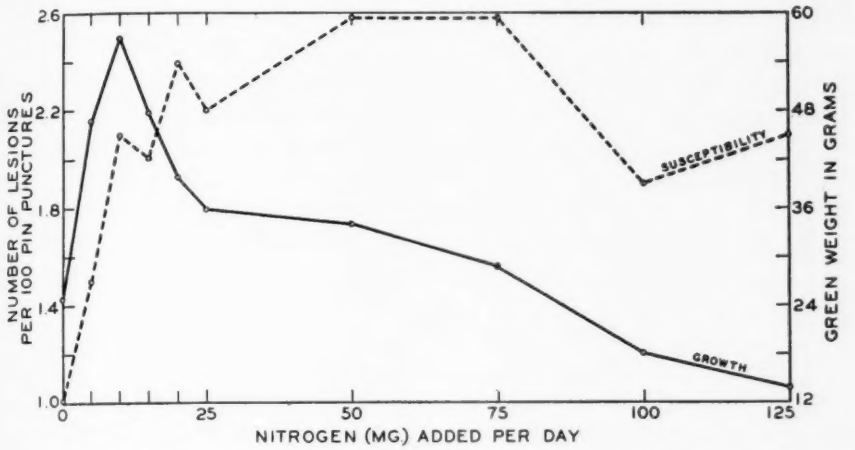

FIG. 6. Influence of nitrogen supply on growth and susceptibility of tobacco to yellow tobacco mosaic when the calcium supply is held constant and only nitrogen is varied. The solid line shows the average green weight at time of inoculation; the broken line, the number of lesions per 100 pin punctures. Five plants were used in obtaining data for each point on the graphs.

TABLE 4.—*The susceptibility of various portions of tobacco plants as influenced by nitrogen*

Nitrogen added per day (mg.)	Number of lesions per 100 pin punctures[a]			
	Upper portion	Middle portion	Lower portion	Entire plant
0	4.3	1.7	1.3	2.1
5	8.7	3.6	1.7	3.8
10	10.1	3.0	2.0	4.1
15	10.5	2.8	1.6	4.0
20	11.8	3.5	2.0	4.6
25	10.9	3.8	2.4	4.7
50	11.8	4.2	2.9	5.3
75	11.1	6.2	3.7	6.3
100	9.0	5.8	4.6	6.2
125	8.4	4.7	3.4	5.2

[a] Each value represents the average of 15 plants.

oculated with yellow tobacco-mosaic virus. Each point on the graphs represents the average value obtained with 5 plants. The graphs in figure 6 correspond very closely with those in figure 5. This similarity indicates, therefore, that the trend of the graphs in figure 5 is due primarily to nitrogen variation and not to variation in the amount of calcium.

It is of interest to note the extent to which the susceptibility of leaves of various ages on the same plant is affected by varying the nitrogen supply. In table 4 are recorded data showing the susceptibility of leaves of the upper, middle and lower portions of the plant at the various nitrogen levels. The results show that the susceptibility of all the leaves on the plant is altered by changing the nitrogen level. The maximum susceptibility in the upper portion is reached at a lower nitrogen level than is reached by the middle portion, and maximum susceptibility in the lowermost portion is reached only at a still higher level. It is also apparent that the upper leaves at any nitrogen level are markedly more susceptible than the middle leaves, and these in turn are more susceptible than the lowermost leaves. There is no change in this relationship between leaves at any nitrogen level tested.

DISCUSSION

The importance of salt nutrition in affecting the susceptibility of plants to bacterial and fungal diseases has been emphasized by many investigators. In a brief review of the literature on this subject, Raines (15) has pointed out that a general correlation exists between host vigor and susceptibility. In other words, a vegetative host is more susceptible to parasitic infection than a nonvegetative or dormant host. The experiments reported in this paper do not extend this general conclusion to the viruses tested.

Experiments on 3 different hosts, one of which is widely separated taxonomically from the family Solanaceae, show quite definitely that the correlation between host vigor, as measured by the rate of growth, and host susceptibility to virus infection may be changed by the nitrogen nutrition of the host. That is to say, with 15 mg. of nitrogen per day, an amount that produced the best growth of tobacco, the plants were only 2/3 as susceptible as they were when 5 times as much nitrogen was added. At this high nitrogen level, however, growth had fallen off nearly 66 per cent. Therefore, in order to get high susceptibility, more nitrogen than is required for good growth must be added. This same relation, although varying slightly in degree, depending on weather conditions and season, was found to hold true with both *Nicotiana glutinosa* and bean. Each experiment was repeated one or more times and not a single exception was noted in any of the tests.

Some other factor, apparently, is more effective than rapidity of growth

in determining the incidence of infection. In the light of present knowledge, it is impossible to advance any satisfactory explanation as to the nature of this secondary factor, but it undoubtedly is related in some way to the physiological processes going on within the living plant cell.

Comparatively little is known with certainty regarding the type of action that nitrogen exerts in regulating host susceptibility to virus infection. Since young tissue is more susceptible to virus infection than old tissue, it is conceivable that cell activity may be a factor for successful virus infection. A study of the nitrogen metabolism within the host may shed some light on the way in which nitrogen influences host susceptibility. It has been shown by Archbold (2) that cell activity as indicated by respiration is relatively rapid when the content of assimilated nitrogen is high. Thus, the increase in infectivity with increasing amounts of nitrogen may be due to an increase in cell activity. It is also conceivable that susceptibility to virus infection may be dependent upon a high concentration of some one of the organic nitrogenous fractions within the plant.

The most generally accepted view is that the virus particle can enter the plant only through an injured cell that is able to recover. Since this repair process calls for synthesis of new protoplasmic material, a deficiency of any substance or element required in this process would indirectly lower the susceptibility of the host. The presence of such a material may be more dependent on some physiological condition within the plant than on host vigor itself.

Further work is necessary to show why growth falls off with increasing amounts of nitrogen. Since cell activity is increased by high nitrogen, it is possible that the carbohydrates in the plant tissue may be used up in respiration before they can be utilized in protein formation. Furthermore, it is possible that protein synthesis may be stopped at some stage in the process by high nitrogen nutrition.

SUMMARY

A study was made of the effect of nitrogen supply on host susceptibility to virus infection.

Turkish tobacco plants grown in soil at different nitrogen levels were inoculated with yellow tobacco-mosaic virus (Johnson's tobacco virus 6) by the pin-puncture method. Susceptibility was measured by the number of lesions per 100 pin punctures. Plants of *Nicotiana glutinosa* L. grown under similar conditions were inoculated with ordinary green tobacco-mosaic virus (Johnson's tobacco virus 1).

In similar tests, Early Golden Cluster beans were grown at various nitrogen levels in sand cultures and inoculated by rubbing the leaves with green tobacco-mosaic virus. Susceptibility was measured by the average number of primary lesions per leaf.

Results from the study of these three hosts indicate quite conclusively, first, that there is a definite correlation between host nutrition and host susceptibility to virus infection, and secondly, that host susceptibility is not governed mainly by host vigor as judged by the rate of growth, but by some other limiting factor the nature of which is at present unknown.

Plants which made the most rapid growth were considerably less susceptible than those in which growth was retarded by excess nitrogen. Thus, in order to get highest susceptibility more nitrogen than is required for good growth must be added. Small additions of nitrogen increase plant growth more than plant susceptibility.

In tobacco the susceptibility of all the leaves on the plant is altered by changing the nitrogen level. The upper leaves at any nitrogen level are markedly more susceptible than the middle leaves, and these in turn are more susceptible than the lowermost leaves. There is no change in this relationship between leaves at any nitrogen level tested.

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ANTISEPTIC SOLUTIONS AND ANTISEPTIC ADHESIVE TAPE IN RELATION TO CONTROL OF HAIRY ROOT, CROWN GALL, AND OTHER OVERGROWTHS ON NURSERY APPLE TREES[1]

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INTRODUCTION

The control of the various types of overgrowth that occur at the union of piece-root apple grafts has challenged the attention of plant pathologists for a number of years. Certain distinct progress in this direction has been made by various writers whose work is reviewed by Riker *et al.* (8). In this paper attention has been directed to the advantages of an adhesive-tape wrapper as one of the most promising means of reducing the percentage of both parasitic and nonparasitic graft knots at the union. As already pointed out (7), (8), certain failures of this tape to provide fully adequate control were found to be either because of the incidence of infection at the time of grafting, or because of the initiation of infection after the wrapper had disintegrated during the latter part of the first year, or during subsequent years in the nursery. Obviously the situation could be influenced little except by reducing the source of inoculum through manipulations during grafting time. The prevention of infection at the time of grafting, however, provided an opportunity for the application of control measures.

Infection at time of grafting, when it did occur, appeared to come primarily as a result of the invasion of bacteria carried upon the surface of seedling roots. It seemed possible to reduce the contamination by these bacteria in two ways: (a) washing from the seedlings all contaminating soil and treating them with antiseptic as developed by a number of writers, reviewed by Riker *et al.* (8), and (b) wrapping the graft with adhesive tape containing antiseptic. Neither of these methods, however, could provide, under commercial conditions, destruction of absolutely all the bacteria, because they may be harbored within certain openings, such as those left by small dead branch roots. On the other hand, both methods present advantageous commercial procedures in which perfection is not required. This question has been here approached from both of these standpoints.

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. These studies were made in cooperation with Johnson and Johnson through the Crop Protection Institute, and with various members of the American Association of Nurserymen. This work was correlated with that done in cooperation between the Bureau of Plant Industry, U. S. Department of Agriculture and the University of Wisconsin.

The easier procedure, as far as the nurserymen are concerned, is the incorporation of an antiseptic within the adhesive tape, avoiding the use of antiseptic solutions. The incorporation of an antiseptic within the plaster mass presented several rigid requirements: (a) No interference with the adhensive quality of the tape; (b) strength enough to kill bacteria and mold without injury to the callus developed at the union; (c) strength enough to preserve the cloth of the tape for maximum protection without preventing the decomposition of the cloth and consequent girdling of the young apple tree.

The purpose of this paper is to describe the use of antiseptic solutions for cleansing the seedling roots prior to the grafting operation and to explain studies on the development of an antiseptic adhesive-tape wrapper.

The selection of an antiseptic was attended with certain difficulties. It seemed desirable first to make laboratory trials of the efficiency of the various antiseptics in common use by plant pathologists and others in relation to their toxic action on *Phytomonas rhizogenes* R. B. W. K. and S. This organism was selected because it is so much more important in the apple nursery than *Phytomonas tumefaciens* (Smith and Town.) Bergey *et al.* The difficulty involved in incorporating an antiseptic into the plaster mass of the adhesive tape made it seem important to approach the problem in two ways: (a) the treatment of artificially infested seedling apple roots with antiseptic solutions, and (b) the introduction of antiseptics into the plaster mass. In addition it appeared that the concentration of the antiseptic employed was of as great importance as the kind. It was not possible to include all kinds of antiseptics in the plaster mass because of certain chemical reactions that interfered with the adhesive qualities of the material, and because certain antiseptics were too volatile.

The basic principles involved in these studies are quite old. The foundation upon which the superstructure of modern surgery rests was stated by Sir Joseph Lister (5) and may be summarized as follows: Decomposition in a wound and affections due to decomposition are intimately connected with microorganisms coming from without. The wound treatment and dressing should prevent the access of microorganisms, and when these have entered, notwithstanding every care, they should be killed or rendered incapable of harm. The substances used for killing microorganisms should not irritate the wound at all, or at least, very little.

One of the writers (Kilmer) was impressed by the striking way in which these processes outlined for the practice of modern surgery have been applied to horticultural practice, as explained in detail in the following pages. While the nurseryman cannot in practice surround himself with the safeguards available to the surgeon, he can in some measure employ the same principles. The nurseryman can apply cleanliness and antiseptics and can avoid infectious particles.

The evolution of Listerism, as exhibited in modern surgery, has bestowed incalculable benefits upon mankind. While the methods herein outlined will not produce such heroic results, it is considered that their study, extension, and practical application will redound to the benefit of horticultural art.

ANTISEPTICS IN SOLUTION

The antiseptic solutions employed are considered first. Since it is well known that the action of an antiseptic against bacteria in a water solution may be greater than when in the presence of tissue, these trials were conducted on seedling apple roots under conditions simulating those found in the nursery. Seedling roots were washed free from surface soil and cut into pieces 3 to 4 inches long. These pieces were soaked from 2 to 4 hours in a suspension of the hairy-root bacteria. The roots were then submerged for varying periods (1, 5, and 10 minutes, respectively, in different tests) in the chemical solutions. After this treatment they were placed in sterile tubes. Doubtless longer treatment would have been more effective in certain cases. The roots were kept at approximately 12° C. for several days, and then small portions (about 3 mm^2) of the surface tissue of each root were removed, placed in Petri dishes, respectively, macerated, and flooded with agar. If any bacterial colonies developed, their characters were examined and their number recorded.

The concentrations of the antiseptic solutions employed were found to influence the results very considerably. Since the examination of the tissues was delayed for several days after their treatment, there was opportunity to determine whether the toxic action of the antiseptic had been injurious to the seedling root. When such injury did occur, it was conspicuous because of discoloration. The injury to the seedling roots was also checked by subsequent examination of their ability to produce callus tissue under favorable conditions. Silver nitrate and mercuric cyanide were, in some concentrations, very effective against the bacteria, but appeared to be injurious to the roots. Except for copper sulphate, none of the other chemicals, as employed, appeared to produce a harmful effect on the root tissue. Mercuric chloride solution, 1:1000, killed all the bacteria. The next in efficiency was cadmium chloride, 1:100. These tests were repeated from 2 to 20 times and a number of them are summarized in table 1.

Various other well-known antiseptics, such as formaldehyde and sodium hypochlorite, were omitted from the preliminary surveys because such volatile substances could not be incorporated satisfactorily into the plaster mass of the adhesive tape. The efficiency of the mercuric chloride, 1:1000, with which many of the nurserymen were familiar in connection with seed-potato treatment, made further study in this direction unnecessary.

TABLE 1.—*Summary of trials to determine the effect of various antiseptic solutions both on apple seedling roots, and on hairy-root bacteria on their surface*

Chemical	Concentration	Repetitions of tests[a]	Roots yielding bacteria	Killing of bacteria	Injury to roots
		No.	*No.*	*Per cent*	
Mercuric chloride	1:250	3	0	100	0
	1:400	3	0	100	0
	1:1000	20	0	100	0
	1:2000	3	8	47	0
Cadmium chloride	1:100	10	0	100	0
	1:200	10	7	86[b]	0
	1:400	9	6	87	0
	1:800	6	9	70	0
Ferric ammonium citrate	1:50	3	10	33	0
	1:100	9	40	11	0
	1:200	9	45	0	0
	1:400	8	39	2	0
Sodium salicylate	1:50	3	10	35	0
	1:100	6	28	7	0
	1:200	6	26	13	0
	1:400	3	15	0	0
Phenol	1:100	3	10	33	0
	1:200	6	25	16	0
	1:400	6	26	13	0
Organic mercury[c]	1:100	5	6	76	0
	1:200	3	4	73	0
	1:400	6	11	63	0
Silver nitrate	1:500	3	0	100	+
	1:1000	5	0	100	+
	1:2000	3	15	0	+
Thymol	Saturated	3	15	0	0
Hydrogen peroxide	Commercial	3	15	0	0
Mercuric cyanide	1:500	2	0	100	+
	1:1000	9	1	98	+
	1:2000	3	12	20	0
Copper sulphate	1:50	4	8	60	+
	1:100	4	12	40	+
	1:200	4	16	20	0
Bordeaux mixture	2:2:50	2	10	0	0
	4:4:50	2	10	0	0
	8:8:50	2	10	0	0
Hexyl-resorcinol	Undiluted	4	19	5	0
	1:1	4	19	5	0
	1:2	4	19	5	0
Control[d]		15	75	0	0

[a] Five roots were used in each test.

[b] In one of these tests, perhaps through error, the results gave a low figure. If this be disregarded, then the percentage of killing effect of bacteria would be 93 instead of 86.

[c] Hydroxymercurichlorophenol sulphate.

[d] Roots soaked in bacterial suspension and then dipped in water. The bacteria were covered in every case.

The results of field tests are reported in later pages.

Certain disadvantages appear in connection with the commercial use of antiseptic solutions on seedling roots such as: (a) Extra expense is involved for the labor, equipment and materials; (b) the seedling roots must be dried after treatment in such a manner that they are not injured; (c) the concentration of the antiseptic must be weak enough to avoid injury to the roots; (d) the strength of the solution must be maintained at a suitable point or it may do more harm than good by spreading the bacteria (8).

Parallel to these laboratory studies tests were made with certain adhesive tapes into which different antiseptics in various concentrations, respectively, were incorporated.

ANTISEPTICS IN ADHESIVE TAPE

The composition of the adhesive mass employed for spreading on muslin was similar to that in common use. The formula, as supplied by the manufacturer, was as follows: gum elastica, 30 per cent; resin from *Picea excelsa*, 15 per cent; resin from *Pinus australis*, 15 per cent; gum olibanum, 10 per cent; and wax, 5 per cent. The remainder was principally a vegetable filler, such as powdered orris root. Various antiseptics were worked into this material before it was spread on the cloth. In each case the concentration is given in percentage by weight.

Different antiseptics or insect repellents were included in the plaster mass in preliminary experiments and were mixed mechanically with the plaster at the time it was prepared. The character of this plaster mass is such that it cannot be mixed by hand, especially if a uniform product is desired. Among the chemicals included in the early tests were phenol, boric acid, thymol, salicylic acid, mercuric iodide, two organic mercury compounds, and nicotine.

BACTERICIDAL ACTION OF ANTISEPTIC TAPE

The antiseptic property of various tapes was tested in preliminary experiments in which the tape was placed in a sterile moist atmosphere and suspensions of the hairy-root organism were smeared over the surface. It became obvious in early experiments that the antiseptic in the adhesive tape was quite different in its action from that of the same antiseptic in solution. Since the plaster mass is relatively impervious to moisture, the effect of any antiseptic in the plaster mass was, naturally, very considerably reduced in intensity. In these preliminary trials it appeared that the bacteria were killed in 1 hour on the mercuric-iodide tape, in 3 hours on the salicylic-acid tape, in 2 days on phenol tape, and in 8 days on boric-acid and thymol tape. They were still alive on nurseryman's tape and on glass slides after 8 days. Since these preliminary tests involved relatively

large masses of bacterial slime, an effort was made to secure a more delicate test against a relatively smaller number of organisms.

Better indications of the toxic action of the antiseptic tape were secured by placing discs (about 8 mm. in diameter) of the antiseptic tape in the bottom of Petri dishes and pouring melted agar, seeded with hairy-root bacteria, over the surface. After a suitable incubation period the relative width of the clear zone about the antiseptic tape disc provided a more delicate index of the antiseptic value of this material. In the preliminary tests the average width in millimeters of the clear bacteria-free margins around the several discs of tape containing antiseptic were as follows: cresol (1:10), 6.5; mercuric iodide (1:100), 5.0; salicylic acid (1:5), 3.0; boric acid (1:10), 2:5 phenol (1:10), 2.0; organic mercury (1:1000), 1.5; thymol (1:20), 1.5; surgeon's 1.5; hydroxymercurichlorophenol sulphate (1:100), 1.2; hydroxymercurichlorophenol sulphate (1:500), 1.0; Graftex, 1.0; nicotine (1:100), 1.0; nurseryman's, 0.0. Although there was no clear area around the disc of nurseryman's tape, there was a small space about 4 mm. in diameter directly above the disc of tape that was apparently free from bacterial colonies. These tests were twice repeated in triplicate with similar results.

TABLE 2.—*Summary of experiments on the toxic effect of various antiseptic tapes upon the growth of hairy-root bacteria in Petri dishes containing bacterial suspensions of varying density*

Antiseptic in tape	Concentration of antiseptic	Margin of clear area around disc of tape in plates seeded with approximately			
		5000 bacteria	10,000 bacteria	20,000 bacteria	500,000 bacteria
		mm.	*mm.*	*mm.*	*mm.*
Mercuric chloride	1:100	36.75	35.50	30.50	10.25
	1:200	35.00	34.25	28.00	10.00
	1:400	29.75	25.25	17.00	9.50
	1:800	19.75	18.00	15.00	7.75
Cadmium chloride	1:100	7.50	6.50	5.50	3.50
	1:200	7.50	6.25	5.00	3.00
	1:400	6.00	5.25	4.00	2.50
	1:800	4.25	3.50	3.00	2.50
Phenol	1:100	3.75	3.00	2.00	2.75
	1:200	3.75	2.75	.....	2.50
	1:400	2.75	2.00	1.50	1.50
Salicylic acid	1:50	5.00	2.75	1.50	.....
	1:100	3.25	2.50	.....	.....
	1:200	3.00	2.25	1.50	.....
	1:400	2.25	2.00	1.00	.....

The concentration of the bacteria in relation to its effect on the width of the bacteria-free margin about the adhesive tape discs was tested. Tape containing mercuric chloride and cadmium chloride in various concentrations were employed in plants seeded with bacterial suspensions in varying amounts. The results of these tests are shown in table 2, where it appears quite conspicuously that the larger the number of bacteria, the smaller the width of the bacteria-free zone. Because of this factor, the concentration of the bacteria in the various comparative tests was made as uniform as possible.

Field tests also were made with these tapes. The results are omitted because of their volume.

In further tests, because of the promise they had given in several directions, only mercuric chloride and cadmium chloride were employed. These studies, run in triplicate and repeated three times, gave results that confirmed those already mentioned. From these results it appeared desirable to examine tapes containing these chemicals in various concentrations applicable to nursery practices.

The bacterial action of these antiseptic tapes was further tried by wrapping them on roots, stems, and grafts of apple plants that had been previously inoculated with heavy suspensions of the hairy-root bacteria. Isolations were made from the surface tissues at various intervals after the treatment. Some of these tests were made in the laboratory, while others were conducted in the greenhouse and the field. The results of the laboratory trials, in which isolations from heavily inoculated and wrapped surface tissues were made about 2 weeks after treatment, showed that mercuric chloride tapes 1:100, 1:200, 1:400, and 1:800 killed bacteria to the extent of 100, 87, 73, and 73 per cent, respectively; and cadmium chloride tapes 1:100, 1:200, 1:400, and 1:800, to the extent of 100, 87, 73, and 66 per cent, respectively. The results of further tests in the greenhouse and in the field, summarized in table 3, showed that the bacteria were killed by the antiseptic tape in a relatively short time after the treatment. Isolations were attempted after various times with negative results. Since the bacteria were killed upon the surface, it seemed desirable to determine whether or not the antiseptic action of the tape would have any influence within cuts made in the apple tissue resembling those produced in grafting.

The extension of antiseptic action from the antiseptic tape into cuts made in apple tissue was studied in experiments similar to those mentioned above except that the stems or roots were cut approximately halfway through before the application of the bacterial suspension. Grafts whose cut surfaces were treated with bacterial suspension before planting in the field were also used. Under such conditions it appeared more likely that the bacteria might be protected against bactericidal action from the anti-

TABLE 3.—*Summary of tests to determine the longevity of hairy-root bacteria smeared on the surface of roots or scions of apple grafts and covered with various antiseptic tapes*

Treatment		Roots or scions[a] from which hairy-root bacteria were recovered after				
		1 day	4 days	12 days	20 days	60 days
		No.	*No.*	*No.*	*No.*	*No.*
IN GREENHOUSE						
Bacteria applied						
Tape wrappers:						
Mercuric chloride	1:100	0	0	0	0	0
Do	1:200	0	0	0	0	0
Do	1:400	0	0	0	0	0
Cadmium chloride	1:100	1	0	0	0	0
Do	1:200	2	1	0	0	0
Nurseryman's tape		3	3	2	3	2
No wrapper		3	2	3	3	2
No bacteria applied						
No wrapper		0	0	0	0	0
IN FIELD						
Bacteria applied						
Tape wrappers:						
Mercuric chloride	1:100	.....	.....	0	0	0
Do	1:200	.....	.....	0	0	0
Do	1:400	.....	.....	0	0	0
Cadmium chloride	1:100	.....	.....	0	0	0
Do	1:200	.....	.....	0	0	0
Nurseryman's tape		.....	.....	2	2	2
String		.....	.....	3	3	3
No bacteria applied						
String wrapper		.....	.....	0	0	0
Nurseryman's tape wrapper		.....	.....	0	0	0

[a] Three employed in each test.

septic tape. On the other hand the spread of the antiseptic through the agar in the Petri-dish tests suggested that a certain amount of spread might take place also into the apple tissue. Since the greatest danger of infection in the apple graft is from the introduction of bacteria into the cortical tissue, it appeared that the antiseptic need not necessarily penetrate far into the wound in order to be relatively effective. The results of these tests are recorded in table 4. As was to be expected, the bactericidal action in the cut was very much slower than that on the surface of the apple tissue. However, it appeared after some time that the possibility of recovering the bacteria from such cuts was very greatly reduced. Further evidence along

TABLE 4.—*Summary of tests to determine the longevity of the hairy-root bacteria smeared into cuts made in apple roots and stems and in the unions of grafts wrapped with various adhesive tapes*

Treatment		Roots, stems, or grafts[a] from which hairy-root bacteria were recovered after				
		1 day	4 days	12 days	20 days	60 days
		No.	*No.*	*No.*	*No.*	*No.*
IN GREENHOUSE (cuts in roots and scions)						
Bacteria applied						
Tape wrappers:						
Mercuric chloride	1:100	3	2	2	0	0
Do	1:200	3	2	2	1	0
Do	1:400	3	3	3	2	0
Cadmium chloride	1:100	3	2	3	2	1
Do	1:200	3	3	3	2	1
Nurseryman's		3	3	3	3	3
No wrapper		3	3	3	3	3
No bacteria applied						
No wrapper		0	0	0	0	0
IN FIELD (graft unions)						
Bacterial applied						
Tape wrappers:						
Mercuric chloride	1:100	.....	.....	3	0	0
Do	1:200	.....	.....	3	1	0
Do	1:400	.....	.....	3	2	0
Cadmium chloride	1:100	.....	.....	3	1	1
Do	1:200	.....	.....	3	2	2
Nurseryman's		.....	.....	3	3	3
String		.....	.....	3	3	2
No bacteria applied						
String wrapper		.....	.....	0	0	0
Nurseryman's tape wrapper		.....	.....	0	0	0

[a] Three employed in each case.

this line is considered later in connection with the hairy-root developed on grafts inoculated and wrapped with antiseptic tape.

In the previous experiment the question arose as to whether the mercury was actually penetrating into the apple tissue, perhaps assisted by surface films of moisture. This question was approached experimentally by making analyses of cortical graft tissue that had been kept wrapped for a few weeks with tape containing mercuric chloride. The surface of the grafts was cleaned with ether to remove any of the plaster mass that adhered after the removal of the tape. These examinations were performed by Eugene Schoeffel under the direction of Professor K. P. Link, who found that by

using the method of Lamholt and Christiansen (3) he was able to secure traces of mercury from this cortical tissue. The amounts of mercury in 0.5 gm. of dried tissue were as follows: Wrapped with nurseryman's tape, 0.00 mg.; with 1:400 mercuric chloride tape, 0.39 mg.; with 1:200 mercuric chloride tape, 0.73 mg.; and with 1:100 mercuric chloride tape, 2.85 mg.

Stand of Grafts Wrapped with Antiseptic Tape

The effect of antiseptic tape upon the growth of callus tissue at the graft union was a matter of some concern, since it appeared (1) that the antiseptic action was strong against the bacteria, (2) that this action penetrated into cut surfaces, and (3) that the tissue apparently absorbed traces of mercury.

The growth of grafts wrapped with tape containing various antiseptics was tried in the greenhouse to see what influence this tape would have upon the stand secured.The results of these greenhouse tests indicated that the kind of antiseptic tape might influence very considerably the stand of grafts secured. While there was considerable range in results in the greenhouse tests with relatively high experimental error, still it was obvious that some of the tapes had sufficient promise to justify more extensive field experimentation.

Duplicate tests with several antiseptic tapes were made in the field (Table 5). Here it appears also that some of the tapes killed off the grafts, while others had promise from the standpoint of antiseptic action without deleterious effects upon the stands. Similar tests, involving about 25,000 trees wrapped with mercuric chloride or cadmium chloride tape, are considered later in connection with the prevention of graft knots. These experiments indicate quite clearly that while sufficient antiseptic may be included to kill off the grafts, the amount may be so adjusted as not to reduce the stand.

A number of factors are involved in this question of stand and wrapping with adhesive tape. Among the more important are the following: (1) The adhesive tape forms a relatively impervious coating over the cut surface of the graft union, which prevents the absorption of moisture by callus at this point. Under conditions of drought in the early spring, tape-wrapped grafts may not secure moisture so easily as those wrapped with string. This consequence occurs very rarely. (2) The mechanical action of the tape as a binder about the union prevents the formation of excess callus at the surface. The callus formation is forced between the cuts of the scion and the root, thus providing a more perfect union. (3) The tape prevents the entrance of contamination from the exterior after the graft is made and, particularly when an antiseptic is present, reduces the activity of molds, which sometimes may cause damage when other wrappers are employed.

TABLE 5.—*Summary of results taken in 1929 and 1930 of experiments that compare the effect of various antiseptic tapes upon the stand of apple grafts planted at various localities*

Location of experiment	Tape employed	Variety of apple graft used	Grafts wrapped	Stand
				Per cent
Missouri	Phenol	Rome Beauty	300	96
	Boric acid	do	300	67
	Mercuric iodide	do	300	79
	Sodium salicylate	do	300	23
	Nurseryman's	do	1000	92
Minnesota	Phenol	Wealthy	250	27
	Boric acid	do	250	26
	Mercuric iodide	do	250	90
	Sodium salicylate	do	250	8
Kansas	Mercuric iodide	Yellow Transparent	500	0
	Cresol	do	440	1
	Organic mercury[a] (1:100)	do	420	97
	do (1:500)	do	500	91
	Surgeon's	do	500	78
	Nurseryman's	do	500	93

[a] Hydroxymercurichlorophenol sulphate.

(4) The tape wrapper makes the union relatively solid, so that there is comparatively little chance of loss from breaking of grafts incident to manipulation in storage and planting operations. (5) If too many layers of tape, especially of antiseptic tape, encircle the graft at one point, the soil bacteria and fungi are unable to destroy the tensile strength of the cloth before the stems begin to enlarge in caliber, and girdling action may result. When the advantages are weighed against the disadvantages and the averages of a series of tests are taken, it appears, as Riker *et al.* (8) reported, that the ordinary nurseryman's tape does not change materially the percentage of stand secured from the grafts, although it does prevent the loss due to breakage in handling. From the foregoing report on stands, it appears that certain antiseptic tapes, when properly prepared, do not reduce the stand.

Preservation of Cloth by Antiseptics

The possibility of girdling action by the antiseptic tape was a matter of concern. Greenhouse tests were made with various antiseptic tapes, some of which decayed in a satisfactory length of time while others lasted too long to be safe as wrappers for apple grafts. The details of these results are omitted because of their volume. The more promising of the antiseptic tapes were tried in the field and their girdling action was recorded under

the most severe drought conditions. During the period 1930–1933 the greatest amount of girdling was found in small experimental plots in Wisconsin. Mercuric chloride tape 1:100 produced 18 per cent girdling, while mercuric chloride 1:200 produced 5 per cent girdling. Lower concentrations of mercuric chloride and the concentrations of cadmium chloride employed produced no girdling and none was observed with nurseryman's tape or with string wrappers. Because of the importance of this question of the duration of tape, records were taken of girdling action along with the records on the influence of different tapes upon the development of graft knots.

The decay of the adhesive tape after a suitable period should not be stressed so greatly as to minimize the importance of having it last long enough. Some of the varieties of tape that had been employed (8), have been unsatisfactory because the cloth disintegrated too quickly. It is altogether desirable that it should last until the maximum protection is provided in strength at the point of union and against the attack of various root-chewing insects. On the average, cloth that gives way in much less than 12 weeks or fails to give way in more than 15 weeks is relatively undesirable. This time varies considerably in different localities during the same season and in the same locality during different seasons. It may be observed that when the season is dry the apple grafts do not make such vigorous growth and the tape has a longer period of time in which to decay. On the other hand, when the season is wet, the grafts may grow more vigorously, but the tape decays more rapidly. The cloth may still appear to be undecayed when the action of soil fungi and bacteria have reduced its strength practically to zero. Consequently, the question of whether the cloth has decayed or not should be determined by an examination of its tensile strength rather than by its appearance.

CONTROL OF GRAFT KNOT

The controlling effect of various antiseptic tape wrappers was studied in a variety of situations. Certain preliminary studies on inoculated grafts were made in Wisconsin and the results are recorded in table 6. Even when a heavy suspension of pathogenic bacteria was introduced into the cut at grafting time, the antiseptic tape prevented disease development in many cases.

Experimental tests on ordinary commercial grafts were made in 1931, 1932, and 1933. These studies are recorded in table 7, which shows that, as previously reported for nurseryman's tape (8), variations were noted depending upon local conditions. Where no diseases appeared, little control was provided. However, when the infectious bacteria were carried upon the seedling roots, very promising control was secured. It will be observed

TABLE 6.—*Summary of results after one growing season showing the effect of various wrappers on the prevention of hairy root at the unions of piece-root Wealthy-apple grafts, inoculated when grafted*

Treatment	Trees examined	Trees with hairy roots	Healthy trees	Girdled trees
Inoculated when grafted	*Per cent*	*Per cent*	*Per cent*	
Tape wrapper:				
Mercuric chloride 1:100	78	44	56	18
Do 1:200	98	44	56	5
Do 1:400	42	62	38	0
Cadmium chloride 1:100	50	86	14	0
Do 1:200	45	89	11	0
Nurseryman's	119	97	3	0
String wrapper	54	100	0	0
Not inoculated				
Nurseryman's tape wrapper	36	11	89	0
String wrapper	109	3	97	0

that while the results with one strength of antiseptic tape were favorable in one place, the results with another were, the same year, more favorable in a different place. Variations occurred in the same nursery in different years. On the average, it appears that the antiseptic tape containing mercuric chloride was superior to any of the others and that a balance was desirable between the extremes of concentration employed with this antiseptic. From these tests it appeared to the writers that a concentration of mercuric chloride of 1:300 was strong enough to provide the antiseptic action desired, and to preserve the cloth for the desired period. At the same time it was weak enough not to injure the graft union and not to preserve the cloth from disintegration beyond the desired period for protection. Consequently, for the last year's tests, this concentration only was used and was found to be satisfactory under the variety of conditions employed. In these tests, very little disease appeared in the controls. It is obvious, however, that the control measures applied in the form of antiseptic tape could have no influence upon factors that induce the development of hairy root during the latter part of the first year or during later years; except, perhaps, a slight action by the residual plaster mass in repelling chewing insects. In a very few nurseries where the infection of the seedling roots was particularly heavy at the time of grafting, the control of graft knots at the union, while a considerable improvement over that secured with nurseryman's tape, was still not fully satisfactory.

In such cases tests were made with treatments of seedling roots in mercuric chloride solution, 1:1000, which is discussed in an earlier section of this paper, about a week before making the graft. The grafts were

TABLE 7.—*Summary of results from experiments during 1931, 1932, and 1933 on the prevention of graft knots by various wrappers*

Wrapper	Trees examined	Stand	Trees smooth	Trees girdled
Results after one season (1931)[a]	*No.*	*Per cent*	*Per cent*	*Per cent*
String	1000	69	76	0
Nurseryman's tape	900	66	86	0
Cadmium chloride tape 1:400	900	69	89	0
Mercuric chloride tape 1:400	900	67	93	0
Results after two seasons (1931 and 1932)[b]				
String	1355	72	53	0
Nurseryman's tape	1590	67	67	0
Cadmium chloride tape 1:400	1513	69	70	0
Mercuric chloride tape 1:400	1355	67	78	0
Results after one season (1932)[c]				
Mercuric chloride tape 1:100	585	74	94	5
Do 1:200	600	74	94	2
Do 1:400	585	72	94	0
Cadmium chloride tape 1:100	580	62	92	0
Do 1:200	555	71	90	0
Nurseryman's tape	585	78	84	0
Results after two seasons (1932 and 1933)[d]				
Mercuric chloride tape 1:000	1093	70	86	1
Do 1:200	1081	69	86	0
Do 1:400	1233	67	86	0
Cadmium chloride tape 1:100	933	53	81	0
Do 1:200	810	64	81	0
Nurseryman's tape	1249	73	80	0
Results after one season (1933)[e]				
Mercuric chloride tape 1:300	1243	84	97	0
Nurseryman's tape	661	83	95	0

[a] Summary of 4 parallel tests in 4 nurseries in 4 states.
[b] Summary of 3 parellel tests in 3 nurseries in 3 states.
[c] Summary of 7 parallel tests in 6 nurseries in 6 states.
[d] Summary of 5 parallel tests in 5 nurseries in 5 states.
[e] Summary of 6 parallel tests in 3 nurseries in 3 states.

wrapped with nurseryman's tape. The results summarized in table 8 show that such treatment, while it may reduce the stand slightly, aids considerably in reducing graft knots.

The treatment of the seedling roots with mercuric chloride solution, subsequently wrapping them with the antiseptic tape, is being continued, but the results are not yet available. The chief concern about this double treatment relates to stand. This should be a promising method in the relatively few places where hairy root is not controlled by the antiseptic tape alone.

TABLE 8.—*Summary of results taken in 1932 and 1933 on the effect of treating seedling roots with an antiseptic solution before grafting*[a]

Treatment	Trees examined	Stand	Trees smooth
Results after two seasons[b]	*No.*	*Per cent*	*Per cent*
Mercuric chloride, 1:1000	2126	59	93
None	2390	63	65
Results after three seasons[c]			
Mercuric chloride, 1:1000	620	63	81
None	1365	68	72

[a] Grafts made with both treated and untreated roots were wrapped with nurseryman's tape.

[b] Summary of 8 tests in 3 nurseries in 3 states.

[c] Summary of 2 tests in 2 nurseries in 2 states.

The recommendations under average conditions for the control of both infectious and noninfectious malformations at the unions of piece-root apple grafts are as follows: (1) Make the grafts from clean seedling roots that have no water on the surface at the time of grafting, and (2) wrap with antiseptic tape that meets the requirements previously outlined. The reasons for having the surface of the seedling dry are (1) to reduce the introduction of infectious bacteria at the time the cut is made and (2) because the adhesive tape does not adhere to a moist surface. No difficulty has been experienced with excessive desiccation of the seedling roots if they are not permitted to remain in the grafting room an unusually long time. In the relatively few nurseries where difficulty was experienced with hairy root due to heavy infection of the seedlings at the time of grafting, good results have been secured by washing the roots; by treating them in mercuric chloride, 1:1000; and by storing them for a week in clean packing material before grafting and wrapping with nurseryman's tape. The infections that occur during the latter part of the first season and during later seasons in the nursery come primarily following attacks by root-chewing insects or mechanical injuries with cultivators (2), (8), which open the way for infectious bacteria to enter. Banfield (1), Levine (4), and Patel (6) have reported that crown-gall bacteria may persist in the soil for several years. Knots that appear during this time may be reduced by planting the grafts in soil where susceptible crops have not been grown for 4 years, by planting in soil previously manipulated so as to be relatively free from root-chewing insects, and by suitable precautions against mechanical injuries near the crown during cultivation.

SUMMARY

Work already described (Riker *et al.*, 8), on the control of knots at the unions of nursery apple trees grown from piece-root grafts, has been con-

tinued and extended. Attention has been directed primarily against bacteria carried on the apple seedlings—a common source of inoculum.

Various concentrations of twelve antiseptic solutions were employed against hairy-root bacteria on the surface of apple seedlings. Mercuric chloride, 1 to 1000, and cadmium chloride, 1 to 100, were effective in killing all the surface bacteria without visible evidence of root injury.

Several series of antiseptics were incorporated into the plaster masses of the nurseryman's-tape wrappers. Their efficiency was tested against the bacteria in Petri dishes, on seedling roots, in cuts in seedling roots, and in grafts. Various trials were made in the laboratory, greenhouse, and field. The more promising antiseptics were employed in various concentrations for their effect on the bacteria, on the preservation of the cloth, on the stand, and on the prevention of graft knots. Mercuric chloride was the best antiseptic employed. Its concentration of 1 to 300 by weight was the most satisfactory.

Under ordinary conditions commercial control was secured merely by wrapping the graft unions with this antiseptic tape. It appeared superior to ordinary nurseryman's tape.

Under uncommonly severe conditions the best control was secured as follows: (1) A week before grafting the cleaned seedling apple roots were treated for a minute in mercuric chloride solution, 1 to 1000. (2) After grafting, the unions were wrapped with nurseryman's tape. The effect of wrapping antiseptic tape on grafts made with treated roots has not been determined.

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A STILBUM DISEASE OF FIG IN LOUISIANA

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INTRODUCTION

The fig, *Ficus carica*, is one of the few fruits that grow well in the southern part of Louisiana. It is not only the most widely grown fruit in that section of the State, but is probably enjoyed by the greatest number of people, as well. Most of the residents along the lower portion of the Mississippi River, Bayou Tèche, Bayou Lafourche, and other smaller streams have one or more fig trees in the yard, garden, or orchard. While a number of different fig varieties are grown to some extent, the Celeste is the one most commonly used. Many of the fig trees are grown in yards more or less shaded by large pecan, oak, or other trees or by buildings of various sorts. The fig trees growing in these damp, shady places are more susceptible to the attack of certain fungus parasites favored by such conditions. This paper deals with a new fig disease recently observed in this territory.

Diseased fig branches were sent in to the Department of Botany, Louisiana State University, in May, 1932, from Hohensolm (Ascension Parish), Louisiana. These branches were covered with small, round, pink heads borne on short stalks, the heads being composed of small, oval, unicellular hyaline spores. The fungus was obtained in culture quite readily by means of spore dilutions in string-bean agar. Preliminary inoculation tests made on young fig branches (Celeste variety) showed the fungus to be an active parasite. Young, infected fig branches were sent to C. L. Shear, Washington, D. C., who tentatively identified the fungus as *Stilbum cinnabarinum* (Mont.).

While a number of Stilbum species have been described from various sections of the United States, as far as the writer can find out from the available literature, *S. cinnabarinum* has not previously been found in this country. Members of the genus, Stilbum, are widespread over the tropics, in some cases causing destructive diseases, such as the "American coffee leaf disease" and others. Apparently, no Stilbum has been previously reported on species of Ficus.

REVIEW OF LITERATURE

A review of the literature on diseases caused by Stilbum species indicates that there are 2 entirely different fungi that have been called Stilbum by various writers.

According to Ashby (2), Cooke (14), in 1880, described the reproductive bodies of a fungus associated with a leaf and berry spot of coffee, which he

called *Stilbum flavidum*. Some years later (1899), Massee (23) mentioned the coffee disease in Nicaragua caused by *S. flavidum* (Cke.).

In 1903, Kohl (20) proposed that the name of the coffee leaf-disease fungus be changed from *Stilbum flavidum* (Cke.) to *Stilbella flavida* (Kohl). Since that time numerous other investigators have noted a similar coffee disease, which they attributed to either *Stilbella flavida* (Kohl) or *Stilbum flavidum* (Cke.). Among these workers, may be noted Puttemans (30) (1904), Delacroix (17) (1911), Fawcett (18) in 1910 and later, in 1915 (19), Maublanc and Rangel (25) (1913), McClelland (27) (1921), Cook (13) (1926), Ciferri (12) (1926) in the Dominican Republic, Chardon (11), in 1927, in Colombia, and Abbott (1), in 1931, in Peru. In 1913 (25) and later, in 1914 (26), Maublanc and Rangel mentioned the existence of a perfect stage of *Stilbum flavidum,* which they called *Omphalia flavida* (Maubl. & Rangl.). In 1925, Ashby (2) described the reproductive bodies of *S. flavidum* as 2–2.5 mm. in height with heads 0.25–0.33 mm. in diameter. He continued "Many Stilbum heads from pure culture and from leaf and berry spots heve been examined for sporulation, but no spores have been found; the enlarged peripheral cells have been seen to give rise only to hyphae in water and on culture media." Ashby clearly demonstrated the presence of the Basidiomycete stage (*Omphalia flavida*) of *Stilbum flavidum* which had previously been mentioned by Maublanc and Rangel.

In 1907, Bernard (8) described a new fungus disease of tea caused by *Stilbella theae* Bernard. He distinguished this fungus from *Stilbum nanum* by the presence of conidia on the stalked heads. Also, in 1920, Averna-Sacca (5) described a species of Stilbum on cacao, with synnemata bearing spherical heads made up of elliptical, hyaline spores. Averna-Sacca (4) also mentioned *Stilbum nanum* (Massee) variety *gossypina* (Sacca), a similar fungus with spherical spore heads, on cotton. Later, the same author (7), in 1927, described a Stilbum causing a discoloration and rot of roses, the synnemata of the fungus having orange or dark red heads and bearing elliptical, hyaline, thin-wall spores. Staner (35) also described a Stilbum on stems of *Vanilla planifolia,* which had small conidia borne in heads, on reddish stalks. In 1925, Jenkins[1] described a similar Stilbum on rose twigs from Cuba, probably the same fungus as that noted by Rorer (31) from Trinidad. Petch (29) noted the common occurrence of *Stilbum cinnabarinum* on dead Hevea bark in Ceylon. It is evident that this group of Stilbums is entirely different from *S. flavidum*.

Other writers have observed diseases caused by Stilbum species, but many of them did not make their descriptions complete enough to deter-

[1] Jenkins, A. E. (*Stilbum* sp. on rose from Cuba). In Martin G. H. Diseases of forest and shade trees, ornamental and miscellaneous plants in the United States in 1924. U. S. Dept. Agr. Bur. Plant Ind. Plant Dis. Rptr. Suppl. 42: 363. 1925. (Mimeogr.)

mine in which group they belong. In 1898, Massee (24) mentioned *Stilbum nanum* on tea; followed by Delacroix (16) in 1902, Watt and Mann (36) in 1903, and Butler (10) in 1905. Numerous others in later years have mentioned this fungus as being destructive on tea plants. In 1931, Leach (21) mentioned a die-back of tea due to *Stilbella* sp., closely related to *Stilbella theae*. Blanchard (9), in 1928, mentioned *Stilbum* sp. on yerba maté (*Ilex paraguensis*) and Averna-Sacca (3) in 1919 observed *Stilbum* sp. causing a serious disease of pear in Brazil. More recently (1931), Mason (22) described an entomogenous fungus, *Stilbella kervillei* Lindau in England. He noted that the fungus is characterized by synnemata up to 5 mm. in height with yellow, waxy heads 4 mm. in height and 0.36 mm. broad, and branched conidiophores $20 \times 1\ \mu$, with small hyaline conidia of 2 types.

Another paper by Averna-Sacca (6) further complicates the Stilbum problem. According to Ashby (2) he mentions "A leaf, berry, and twig spot disease of coffee in São Paulo, Brazil, associated with the conidial synnemata of a Stilbella having spherical, vermilion spore heads. Although perithecia were not seen, he referred this fungus to *Sphaerostilbe flavida* Mass., citing *Stilbum flavidum* Cke., *Stilbella flavida* Kohl., and *Omphalia flavida* Maubl. and Rangl. as synonyms." Ashby writes "it is evident that the conidial fruiting body described by him (Averna-Sacca) had nothing in common with the Stilbum body of the American coffee leaf disease, and it is not clear from his account that he was dealing even in part with that disease." There are evidently 2 tropical coffee diseases, caused by 2 fungi that are similar in appearance, but quite different when examined closely.

The original description of Stilbum (Tode) as given by Saccardo (33) is as follows: "Stroma tereliusculum apice capitato-conidiophorum ex hypis coalitis conflotum. Conidia minuta continua muco primitus abvoluta." This description indicates quite clearly that there are conidia associated with the fungous heads, which necessarily eliminate the so-called "Stilbum" forms that do not have conidial heads.

Apparently, there have been 2 different organisms called by various writers ether *Stilbum flavidum* (Cke.) or *Stilbella flavida* (Kohl.). They are (1) a true Stilbum, characterized by the production of short synnemata, with small spherical heads composed of large numbers of small, ovate, unicellular conidia, and the (2) "pseudo-Stilbum," originally described as *Stilbum flavidum* (Cke.), which has small spherical heads produced on short stalks very similar in appearance to those of the true Stilbum, but the heads have no conidia associated with them.

The fungus associated with the fig disease in Louisiana is a true Stilbum having the conidial heads characteristic of that genus.

THE FIG DISEASE IN LOUISIANA

Symptoms.—The first evidence of the Stilbum disease on fig is the presence of dead twigs scattered about over the tree. Usually the very youngest twigs are killed back. The small pink fungus heads (Fig. 1, A and B) appear in large numbers on the bark of the dead or dying branches. There may be a definite canker stage when larger branches are affected and sunken areas may appear in large branches at the base of smaller ones. The fungus usually develops much more slowly in the larger branches than in the small twigs. The tissues just beneath the bark of diseased twigs turn a dark brown and this discoloration usually extends from 3 to 10 cm. in advance of the fungus heads, and extends through the wood into the pith. Small boring insects often infest the twigs soon after they are killed and make the destruction complete. Such diseased branches also may be severely affected by winter injury. The fungus may spread into the larger branches and eventually destroy the entire tree, or make it worthless, depending upon whether or not conditions are favorable for its development. On the other hand, many old fig trees have been observed that had a number of dead branches covered with Stilbum heads, but apparently the fungus was spreading very slowly, and causing very slight damage. No Stilbum infection has been observed on either fig leaves or fruit.

Distribution and Importance.—The Stilbum disease has been found scattered over most of the southern part of the State, from Baton Rouge as far west as Lafayette and north to Bunkie. One small infection also was found near Gulfport, Mississippi.

The importance of the disease is rather difficult to estimate. Many fig trees are grown for ornamental purposes as well as for the production of fruit. Any condition that causes the death of a few branches and results in such trees looking ragged and unhealthy is of some importance. Some trees are killed outright and others are so badly damaged that they must be destroyed, while many trees have only a few infected twigs and are not seriously damaged by the fungus.

Most of the damage observed on fig trees in the southwestern section of Louisiana has been found on the Celeste variety. One infection was found on a planting of Magnolia fig trees, but the damage was much less severe on trees of this variety. In one orchard where trees of the Magnolia variety were found surrounded by Stilbum-infected Celeste trees, the former have remained entirely free of Stilbum infection. Since the Celeste is the best quality fig, as well as the most widely grown variety in this section, the disease is of considerable economic importance. It can be safely stated that the Stilbum disease is second in importance to thread blight caused by *Corticium koleroga* (Burt.), among fig diseases in this section of the State.

FIG. 1. *Stilbum cinnabarinum* on fig branches. A. Stilbum heads on an old twig. ×6. B. Naturally infected twigs showing numerous spore heads. Slightly reduced. C. An inoculated twig showing spore heads around point of inoculation. Photograph taken 38 days after inoculation. Natural size.

Environmental Conditions.—It is evident that infection takes place more readily under humid conditions. As was previously mentioned, the Stilbum disease is confined principally to very damp, shady places. Where it has been found in most serious form, the infected fig trees were usually growing in close proximity to large pecan or oak trees, or partly in the shade of certain buildings. But in some cases where inoculations were made on fig trees in the orchard away from any shade trees infection spread to the larger limbs quite rapidly.

Seasonal Development.—Observations made over a period of 2 years indicate that the Stilbum disease develops most rapidly during the late spring and summer, being much more severe during very wet spells. However, some inoculated trees have shown fungus development to some extent during the fall and through most of the winter. Temperature studies have shown that the fungus is favored by warm weather, although it grows slowly at fairly low temperatures.

Source of Infection.—Although the conidia are held firmly in the Stilbum spore heads, they become loosened somewhat when the heads come in contact with moisture. It is probable that wind, splashing rain, and possibly various insects may serve as disseminating agents for the conidia. The results of experimental inoculations indicate that some sort of wound is necessary before infection can take place. Since most of the infections seem to occur at or near the tips of the young twigs, it seemed probable that the fungus might enter through leaf and fruit scars. This theory was verified by a series of inoculations described later in this paper. Infections probably occur also through wounds made by boring insects, as well as through other types of wounds. During the summer of 1933 a number of Stilbum infections were found following wounds made by very large hailstones that fell during a severe storm in May.

The Causal Organism.—The first cultures of *Stilbum cinnabarinum* were obtained by making spore dilutions in string-bean agar from one of the small Stilbum heads. Transfers were made to sterile string-bean pods after 3 days. Growth was rapid and the first inoculations were made with spores from these bean-pod cultures. After the fungus had proved to be parasitic, single-spore cultures were obtained and all later inoculations were made with these.

On nearly all the culture media used this fungus produced single-cell conidia that were exceedingly variable in size (Fig. 2, B). The size varied from $3 \times 1.5\,\mu$ to $15 \times 5\,\mu$. The greatest variation in size of conidia occurred on acid-agar plates. When grown on sterilized fig twigs in large test tubes, some large, dark brown masses of irregular conidia were produced. The spores from these masses—probably sporodochial in character—were quite uniform in size and shape, and typical of those that develop

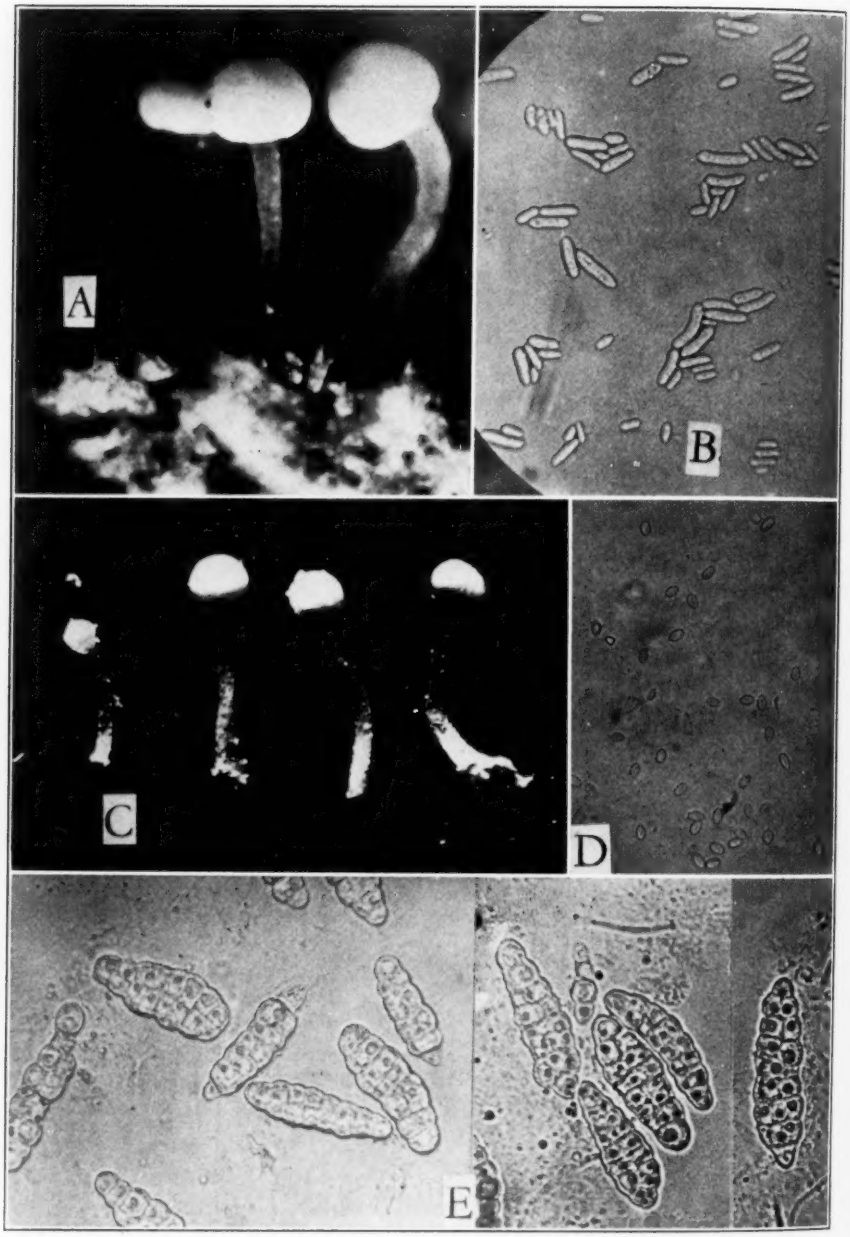

FIG. 2. Stilbum spore heads, conidia and ascospores. A. Stilbum heads showing typical condition. ×30. B. Conidia from pure culture (acidified string-bean agar) showing variation in size. ×386. C. Stalked heads similar to those in A. ×21. D. Stilbum conidia from spore head on infected branch. Typical of those always found under natural conditions. ×386. E. Ascospores of *Megalonectria pseudotrichia*. ×600.

on the stalked heads under natural conditions, the average size of these conidia being $5.7 \times 3.0\ \mu$. Although the fungus has been grown on a large number of different media and under varying conditions of light and temperature, only in a few cases have the stalked heads been produced in culture, and in most cases these did not develop normally.

One strain of the fungus obtained from a germinating ascospore produced the typical Stilbum heads rather consistently when grown on sterile string-bean pods, but they were smaller and more irregular in size and shape than those occurring naturally on fig branches. The conidia produced on these stalked heads were, however, quite uniform in size and shape.

On infected fig branches the Stilbum develops in a different manner from the way it grows in pure culture. The small pink heads break through the epidermis of the twig and the stalks gradually elongate until they reach their full length. The stalks—synnemata—develop either singly or in groups of as many as 4 or 5. Often 2 stalks may be joined together at the top in a single large head. The synnemata are smooth and vary from 50 to 200 μ in diameter and from 1 to 2 mm. in length, tapering slightly toward the upper end, each stalk being composed of numerous septate hyphae, compressed into a very compact mass that flares out somewhat at the top (Fig. 2, A and C). The heads, which are nearly spherical but slightly flattened at the base, are brick-red when first developed. Later the color may fade to a light pink, as the result of weathering. The heads range from 200 to 400 μ in diameter, the average being about 300 μ. The ovate, hyaline, single-cell conidia form a solid, compact mass on the top of the stalk, being held together by a genatinous material. The measurements made of several hundred conidia from different sources showed the size to vary from 3.5 to 7.5×2.5 to 4.1 μ, the average being about $6 \times 3\ \mu$ (Fig. 2, D).

The above description of this fungus fits in fairly well with the original description of *Stilbum cinnabarinum* by Montagne (28) as given by Saccardo (34). The Louisiana form has synnemata somewhat shorter (1–2 mm.) than those described by Montagne (2–4 mm.) and conidia slightly larger, $6\ \mu \times 3\ \mu$ as compared with $5\text{–}6 \times 2\ \mu$. Since these differences seem to be only minor ones, the fungus herein described is considered to be synonymous with *Stilbum cinnabarinum* (Mont.).

Ascigerous Stage of the Fungus.—A few perithecia containing muriform, multiseptate ascospores were found associated with the Stilbum on fig branches about February 1, 1933. Single-ascospore cultures obtained from this material developed the characteristic Stilbum-type conidia in culture, and inoculations with these conidia into young fig branches produced the disease in typical form.

Early in January, 1934, a number of fig branches containing the bright red perithecia were found on Stilbum-infected fig trees in Ascension parish, some 35 miles below Baton Rouge. The perithecia were produced on the lower surface of dead twigs ½ to ¾ inch in diameter, singly or in groups of 2 to 5, often at the base of the old conidial stalks. They were often found attached to the bases of these old stalks. The perithecia when collected were bright red, almost perfectly round, quite soft in texture, and borne on very short stalks. In the moist, fresh condition they measured from 475 to 525 μ in diameter. Older perithecia, which had turned dark brown to almost black, were obtained from other trees. These measured from 300 to 450 μ in diameter and were somewhat sunken at the top. Soon after the round, bright red perithecia were collected (January 15), they contained a few asci with mature ascospores, and after being kept quite moist for a few days, they had considerable numbers of asci with mature spores. There were apparently no paraphyses present. The normal asci (Fig. 2, E), which contained 8 spores, were from 80 to 130 μ in length, and 16 to 20 μ in diameter, averaging 100×17 μ.

The ascospores were found to be very variable in size, ranging from 20 to 40 μ × 9–14 μ. Some ascospores were obtained from normal perithecia that were as long as 52 μ, but these had apparently begun to swell preparatory to germination. The ascospores were hyaline, muriform, with 5–7 transverse septa.

The asci could be forced out of the mature perithecia quite readily when slight pressure was exerted upon them, but the ascus wall itself was difficult to break. Often the spores were completely crushed before the ascus would break open, and the spores often germinated while still within the ascus. The ascospores germinated quite readily in tap water or in string-bean agar, producing numerous small germ tubes. As many as 25 germ tubes were counted on a single germinating ascospore.

Although *Stilbum cinnabarinum* has been grown on a large number of different culture media, including fig agar, and sterilized fig branches, as well as under varying light and temperature conditions, no perithecia have been produced in pure culture. However, perithecia have developed on fig branches inoculated with pure cultures of the fungus the previous year. All the observations made by the writer indicate that the perithecial stage of this fungus develops only in southern Louisiana under the most favorable moisture conditions, and then for a very limited period during January and February.

The perithecial stage of *Stilbum cinnabarinum* has been mentioned by Petch (29) as *Megalonectria pseudotrichia* (Schw.) Speg. The perithecia were said to occur along with the Stilbum heads on dead Hevea bark in Ceylon. The perithecial stage of the Louisiana Stilbum resembles

Megalonectria pseudotrichia (Schw.) Speg., as described by Saccardo (32), quite closely in most respects. However, the closely related form *Megalonectria nigrescens* (Kalch. & C.) Sacc. or *Sphaerostilbe nigrescens* (Kalch. & C.), as described by Cooke (15), also resembles the Louisiana form. Since the perithecial stage of the *Stilbum cinnabarinum* has been previously described by Petch as *Megalonectria pseudotrichia* (Schw.) Speg., the Louisiana form is tentatively listed under the same name.

Temperature Relations.—Two Stilbum cultures were used in temperature studies: (1) the original isolation, which had been used in most of the inoculation tests, and (2) a new culture isolated from another source in the fall of 1933. Both of these were monosporous cultures, known to be actively parasitic on fig branches.

Petri dishes of equal size, containing equivalent amounts of agar (string-bean and dextrose (Difco) agar), were inoculated in the center with small square pieces of agar from a rapidly growing culture of the fungus. They were kept at room temperature until the fungus started growth and were then placed in a series of incubators ranging from 6 to 37° C. Measurements were made of the diameter of the colonies (5 plates of each culture at each temperature) at 24-hour intervals, until the colonies covered the plates at the optimum temperatures. Growth was quite similar on both media with both cultures. There was some growth over the entire range of temperatures but very little at the extremes. The average growth in diameter of the colonies in mm. per 24-hour period was taken as a criterion of comparison. Growth was quite slow at 6° and 37°, the optimum temperature for most rapid growth being 28–30°, while the growth curve falls very rapidly between 32° and 37° C.

Pathogenicity.—The preliminary inoculation tests were made on fig trees of the Celeste variety in the vicinity of Baton Rouge, in June, 1932. Young, rapidly growing branches of the current year's growth were inoculated by making a small slit in the bark, inserting a small portion of a string-bean pod culture of the Stilbum into the wound, and sealing the slit with melted paraffin. A corresponding number of twigs were wounded and pieces of sterile bean pod inserted into the slits as controls. When examined 22 days later, 6 of the 10 inoculated twigs had some of the Stilbum heads developing on the bark around the point of inoculation. Two weeks later, several of the twigs were dead above the wound and the fungus had developed over the surface of the dead twigs. The controls all remained healthy.

The fungus was reisolated from these inoculated twigs and another set of inoculations made with the newly isolated cultures on August 2 of the same year. After 36 days, 6 of the 15 inoculated twigs were dead and the other 9 showed reddening of the bark, and some fungus growth around the point of inoculation. Later all of this lot of twigs were killed outright.

An inoculated twig with spore heads of the fungus is shown in figure 1, C. On February 17, 1933 (about 6 months after inoculation), the fungus had spread on 2 of the twigs to a point about 2 feet from the inoculation so as to kill back the 2-year-old wood for several inches. About a year later (18 months after inoculation), 2 main branches, 2 inches or more in diameter, had been killed back to the main trunk. This series of inoculations, along with several other more extensive ones, indicates that this Stilbum may be quite destructive under certain conditions.

In September, 1932, another set of inoculations was made on trees of the Celeste variety to determine more definitely how infection may take place. A known parasitic culture of Stilbum growing on sterile bean pods was used for inoculum. There were 4 sets of inoculations with appropriate controls, each consisting of 10 twigs: Lot 1 was inoculated in the usual way by means of a small slit in the bark of young twigs of the current year's growth; Lot 2, 2 leaves were broken off near the tip of each small twig and small portions of inoculum placed on the scars left after the removal of the leaves; Lot 3, 2-year-old wood inoculated by wounding in the usual way; and Lot 4, 2-year-old wood inoculated on the unwounded surface. Thirteen days later in Lot 1, one twig was dead, 3 others had Stilbum heads developed around the wound; while in Lot 2, 9 of the 10 twigs had Stilbum heads developing at the point of inoculation; 2 of the twigs in Lot 3 showed infection, while all those in Lot 4 remained healthy. When next examined on November 8 (60 days after inoculation), 6 twigs of Lot 1 were killed back 6–10 cm.; 7 of Lot 2 killed back 3–7 cm.; 6 of Lot 3 showed dark discolorations 3–5 cm. around the point of inoculation; while all the branches in Lot 4 (unwounded) remained free of infection.

During the following year many of these infections spread into the older branches, killing them back from 1 to as far as 3 feet in some cases, while in other twigs the spread was very limited. Two of the larger inoculated branches were killed back to the main trunk, and the dead wood was covered with the bright colored Stilbum heads. This test showed that the Stilbum does not penetrate the unbroken bark of the fig branch very readily, if at all, and that infection may take place through the leaf scars if the leaves are removed just before they are ready to shed. It seems likely that most of the natural infection occurring in fig trees may take place through the leaf or fruit scars near the tips of the small twigs. If favorable conditions occur, the infection may spread rapidly into the older branches.

No Stilbum infection has been found on either the leaves or fruit of the fig. Preliminary inoculation tests made on both the green fruit and on leaves have failed to produce infection. It is likely that the Stilbum infection may be confined entirely to the woody portion of the fig tree.

Host Relations.—In the early part of April, 1933, a number of twigs of peach, plum, pear (Pineapple Sand variety), and seedling pecan trees were inoculated with *Stilbum cinnabarinum*. There was no evidence of infection on any of the trees except the pear. The fungus caused rather rapid blackening and dying of most of the inoculated young pear twigs of the current year's growth. Stilbum heads developed in the dead bark of some of the twigs several weeks after the twigs were killed, but they were much smaller, very pale pink, and not nearly so well developed as those produced on fig. There has been no indication of spread of the Stilbum from the young branches into the older portions of the pear trees, as it occurs in the fig.

A preliminary set of varietal inoculations was made in the spring of 1934. The following varieties were used: Celeste, Brunswick, Magnolia, Brown Turkey, and Green Ischa. There were 8 twigs on each of these varieties inoculated with each of 2 strains of *Stilbum cinnabarinum*. Appropriate controls were also used. After 45 days the twigs were examined. The Green Ischa, Brown Turkey, and Magnolia were severely injured, while infection was less severe in the Celeste and Brunswick. While these tests are only preliminary, they indicate that other fig varieties than the Celeste may be susceptible to Stilbum infection.

Since a very similar fungus has been found causing a canker of rose in Cuba, it was thought to be of some interest to make inoculations on this plant with the Louisiana Stilbum. A preliminary set of inoculations made on the climbing rose (Killarney variety) with two Stilbum cultures failed to produce any infection after 3 months, while fig branches inoculated at the same time with the same cultures were killed.

Control Measures.—Pruning out infected branches and painting the cut ends with ordinary outside paint has proved to be fairly effective in controlling Stilbum on fig trees that had only a few incipient infections. A group of 6 fig trees, 8 to 10 years old, on the property of Mr. Reuss (where Stilbum was first discovered in Louisiana) had a few infected twigs on each tree. These diseased twigs were cut out and a coating of heavy gray outside paint applied to the exposed surfaces. A year later, when the trees were examined, only 2 infected twigs were found on the entire group of 6 trees.

However, on an old, heavily infected tree the results were different. Several large diseased branches were removed from this tree and the cut ends were covered with a coating of heavy paint. This was done during the early part of April, 1933. Within 6 weeks the Stilbum heads were very numerous around the cut ends of 3 large branches and the fungus heads had grown directly through the heavy coating of paint in 2 cases. It is probable that these branches had not been cut back far enough to get beyond the infected wood.

While no definite control recommendations based on accurate data can be made at present, it is believed that some precautions can be taken which will reduce the damage caused by Stilbum on fig trees. If the trees are set out in the open, away from the shade of buildings, larger trees, etc., the chances for Stilbum infection are greatly reduced. Pruning out the interior of the trees so as to allow sunlight to penetrate as much as possible is also a good practice. If Stilbum-infected twigs are found, they should be removed, care being taken to cut back beyond the brown discolored bark. Painting the cut ends of the branches may also help to prevent outside infection.

SUMMARY

A new disease of fig, *Ficus carica*, caused by *Stilbum cinnabarinum* (Mont.) is described from Louisiana. Stilbum diseases are prevalent in the tropics, but this is apparently the first one described in the continental United States.

The Stilbum disease is widespread over southern Louisiana, and, under certain conditions, causes considerable damage to fig trees. Diseased branches usually have the characteristic small pink fungus heads, borne on short stalks growing on the bark.

The ascigerous stage, *Megalonectria pseudotrichia*, of *Stilbum cinnabarinum* has been definitely connected with the conidial or Stilbum stage.

Inoculation tests show that the fungus attacks fig twigs readily through slight wounds, but apparently not through the unbroken epidermis.

The Stilbum disease can be controlled in young trees by pruning out infected branches, but old, badly diseased trees should be destroyed.

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RELATION BETWEEN NUMBER, SIZE, AND LOCATION OF SMUT INFECTIONS TO REDUCTION IN YIELD OF CORN[1]

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INTRODUCTION

During recent years in many States severe losses from corn smut (*Ustilago zeae* (Beckm.) Ung.) have emphasized the economic importance of this disease. It is conservatively estimated that 2 per cent of the United States corn crop, or 55,000,000 bushels, is destroyed annually by smut (5). During the 3 years, 1931 to 1933, the losses from corn smut in Minnesota have ranged from 5 to 10 per cent (5).

The damage caused by corn smut is evident when seedlings or young plants are killed, ears are destroyed, or when the stalks are severely infected, but until recent years it has not been generally recognized that the presence of a smut gall on an otherwise apparently healthy plant may materially reduce its yield. Immer and Christensen (2, 3) and Jorgenson (4) found that the reduction in yield of inbred lines and F_1 crosses of corn due to a single smut infection was dependent upon the size and location of the smut gall on the plant. Garber and Hoover (1) found a higher percentage of barren stalks on smut-infected than on smut-free plants but were unable to demonstrate other significant decreases in yield resulting from smut infection.

Only meager information is available regarding the reduction in yield caused by two or more galls on the same plant or by ear infection. Furthermore, little is known regarding the effects of smut on normal varieties of corn.

The present paper summarizes the results on smut losses that have been obtained in Minnesota since the earlier publications by Immer and Christensen (2, 3). The tests were made particularly to determine the reduction in yield caused by two or more galls on the same stalk and by galls located on the ear. Studies on the effects of single smut galls, however, were continued in order to eliminate errors due to seasonal fluctuations and to check the accuracy of earlier investigations. In addition, tests were made on the indirect effect of smut infection on kernel luster and as a predisposing factor for ear rots.

MATERIALS AND METHODS

In 1932 and 1933 more than 1,800 comparisons were made between adjacent smutted and smut-free plants of dent corn consisting of 3-way

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crosses, double crosses, and top crosses; standard open-pollinated varieties and inbred lines were used also. All plants were grown in rows either with single plants spaced 1 foot apart or in hills of 2 to 3 plants. In the drilled corn each paired comparison consisted of a smut-infected and smut-free plant of the same variety, cross, or inbred line growing 1 foot apart; in the hill-planted corn the paired comparison consisted of 2 plants growing in the same hill. Since the hybrids and varieties used in this study were rather variable, only those paired plants that were similar in size were used. The ears from the smutted and smut-free plants were harvested and placed in paper bags, the ears from the smutted plants were marked, and the number, size, and location of the smut galls were recorded. The harvested ears were thoroughly dried at 170° F. and then weighed without shelling.

In 1932 no attempt was made to select pairs of plants with smut galls of specific size or location, but in 1933, in order to obtain a representative population for each gall of a particular size and location, only those pairs were harvested that were obtained with least frequency during the previous year. During both years approximately 600 comparisons were obtained between smut-free plants and plants infected with 2 or more galls; and over 1000 comparisons were obtained between smut-free and smut-infected plants having only a single gall. Statistical analysis of the data was made by Student's pairing method; each pair consisting of an adjacent smutted and smut-free plant.

EXPERIMENTAL RESULTS

The data obtained in this study are most conveniently classified into 3 parts: comparisons of smut-free plants and infected plants with a single smut gall; comparisons of smut-free plants with infected plants having multiple smut galls; and the relation of smut infection to kernel luster and to ear rots.

Single Smut Galls

The relation between yield and infection by small, medium, and large smut galls, at 9 specific locations on the plant, was studied with the varieties and crosses as shown in table 1. In the original analysis of the data, the results for the varieties and crosses were summarized separately for each of the 2 years, but since the varieties and crosses responded in a similar manner and no differences were observed between the 2 years, all of the data were combined to utilize a large number of comparisons.

From the data given in table 1 it is evident that the losses from smut infection are dependent upon the size and location of the smut gall. Small galls on the nodal shoots below the ear did not appreciably reduce the yield, but the losses from medium-size and large galls at the same locations were

TABLE 1.—*Relation between size and location of single smut galls on varieties and crosses to reduction in yield of ear corn*

Location of gall	Size[a]	No. of comparisons	Per cent reduction	Odds that loss is significant
Base	Small	23	2.4	2:1
	Medium	34	15.5	230:1
	Large	10	17.7	7:1
Shoot	Small	113	1.9	2:1
	Medium	119	18.2	250:1
	Large	35	50.2	>5,000:1
Sucker	Small	29	1.9	1:1
	Medium	15	3.3	2:1
	Large	4	26.0	3:1
Total below ear	Small	165	2.0	2:1
	Medium	168	16.5	152:1
	Large	49	41.7	>10,000:1
Main stalk	Small	28	16.3	1,750:1
	Medium	31	39.8	>10,000:1
	Large	25	99.0	>10,000:1
Neck	Small	126	11.2	300:1
	Medium	127	27.1	>10,000:1
	Large	31	75.9	>10,000:1
Tassel	Small	19	7.2	14:1
	Medium	9	88.4	>10,000:1
	Large	3	100.0	112:1
Total above ear	Small	173	11.6	249:1
	Medium	167	33.0	>10,000:1
	Large	59	86.7	>10,000:1
Ear tip	Small	46	−1.1	2:1
	Medium	40	19.4	>10,000:1
	Large	18	52.0	>10,000:1
Ear general	Small	18	10.7	6:1
	Medium	16	28.7	130:1
	Large	94	90.0	>10,000:1
Total ear infections	Small	64	2.2	2:1
	Medium	56	22.0	>10,000:1
	Large	112	83.4	>10,000:1
Leaf	Small	19	−6.2	4:1

[a] Small galls—up to 2 inches in diameter.
Medium galls—from 2 to 4 inches in diameter.
Large galls—over 4 inches in diameter.

highly significant. Small and medium-size galls on the suckers did not reduce yield, although large galls at this location reduced the yield by 26.0 per cent. The total of all single gall infections below the ear, namely, base, shoot, and sucker, caused a weighted average loss of 2.0 per cent for small, 16.5 per cent for medium, and 41.7 per cent for large galls.

Smut galls, particularly the medium and large-size galls, located above the ear were much more destructive than those below the ear, and frequently resulted in barren stalks. The difference in destructiveness of galls located above and below the ear is explained on the basis of the part of the plant

attacked. Infections below the ear were largely on ear buds, while infections above the ear were chiefly nodal or internodal and injured the main stalk.

Smut galls on the tassels[2] gave a surprisingly large reduction in yield. Since only 31 comparisons were obtained, it is not desirable to draw too definite conclusions as to the exact losses. Medium and large galls on the tassel were nearly always accompanied by barren stalks, but small galls caused no significant decrease in yield. Many of the small galls, however, consisted merely of a few scattered spikelet infections. These results are not in agreement with those obtained by Garber and Hoover (1), who found no reduction from tassel infection.

The statistical analysis in table 2 is made by comparing the average reductions in yield resulting from infection by small, medium, and large single galls above and below the ear. Differences of 3.7, 4.0, and 5.6 times the standard error, respectively, prove conclusively that smut galls above the ear caused the greater loss in yield. The data on the relative destructiveness of galls below and above the ear confirm the earlier results obtained by Immer and Christensen (2, 3) and Jorgenson (4).

There were two quite distinct types of ear infections. In one type, the smut gall was attached to the tip of the cob and in the other it was generally distributed over the ear and usually resulted from several kernel infections. These infection types have been designated in table 1 as "ear-tip" and "ear-general," respectively. They were compared separately with smut-free plants, and the results show that on the average the tip infections are less destructive than the general infections. The average yield of 46 plants with small galls on the ear tip was slightly more than that of the smut-free plants, but the difference in favor of the infected plants was not statistically

TABLE 2.—*Average reduction and average difference in yield in grams between paired smut-free and smut-infected plants*

Size of galls	Galls above the ear		Galls below the ear		
	Number of comparisons	Av. reduction in yield	Number of comparisons	Av. reduction in yield	Difference
Small	173	21.18 ± 2.99[a]	165	3.66 ± 3.69	17.52 ± 4.75
Medium	167	60.69 ± 5.14	168	31.81 ± 5.00	28.88 ± 7.17
Large	59	155.85 ± 7.66	49	77.08 ± 11.93	78.77 ± 14.17

[a] Standard errors.

[2] In case of tassel infection a so-called large gall consisted of a large number of closely aggregated spikelet infections; a small gall, of few scattered infections.

significant. Small galls of the general type on the ear gave a reduction of 10.7 per cent, although the difference was not statistically significant. Plants with medium and large galls of the ear-tip or ear-general type yielded significantly less than the smut-free plants used in making comparisons. Large galls, generally distributed, usually destroyed the entire ear, while similar sized galls on the ear tip reduced the yield 52.0 per cent. A statistical comparison between the average reduction in yield from small, medium, and large galls of the ear-tip and ear-general type gave differences that were not significant for small and medium galls but highly significant for large galls. The difference between the reduction in yield caused by general ear infection and ear-tip infection was 4.8 times the standard error.

Over 200 comparisons with single smut galls on inbred lines at different locations gave essentially the same relationship as shown with the varieties and crosses.

Multiple Smut Galls

Under normal field conditions plants with 2 or more smut galls occur quite frequently. At University Farm, in 1933, a study of the smut reaction of 40 varieties and crosses showed that 20–25 per cent of the infected plants had 2 or more smut galls. Since multiple galls may vary in position on an individual plant, the data were classified into 3 groups on the basis of smut locations, namely, those plants on which all galls were below the ear, those on which a part were above and a part below the ear, and those on which all galls were above the ear. Since the number of galls on each plant varied also, the data were classified into 2 groups on the basis of number of galls. This classification includes those plants bearing 2 galls and those bearing 3 or more galls per plant. According to size of galls per plant, the groups were divided into 5 classes, namely, small, small and medium on the same plant, medium (1 large and 1 small gall were classified as 2 medium galls), medium and large on the same plant, and large galls. The average percentage reduction from 600 paired comparisons of smut-free plants and plants with 2 or more galls is given in table 3.

Comparing plants with smut galls of similar size, the results show that infection below the ear gave the least reduction; plants in which a part of the galls were above and a part below the ear were next, and plants with all galls above the ear gave the greatest reduction in yield. Losses in yield from multiple infections are also dependent upon the size of the smut gall. Two small galls per plant below the ear, above the ear, and generally distributed over the plant reduced the yield 12.9, 28.9, and 13.9 per cent, respectively, while medium galls at these locations gave 3 times larger losses in yield. Large multiple galls usually caused complete stalk barrenness regardless of the position of the gall on the plant.

TABLE 3.—*Relation between size, number, and location of multiple smut galls on varieties and crosses to reduction in yield of ear corn*

Number and size of multiple galls[a]	Location of smut galls								
	All below the ear			Above and below the ear			All above the ear		
	Number of of comparisons	Per cent reduction	Odds that loss is significant	Number of of comparisons	Per cent reduction	Odds that loss is significant	Number of of comparisons	Per cent reduction	Odds that loss is significant
S, S	47	12.9	44:1	40	13.9	53:1	19	28.9	>10,000:1
S, S, S+	15	19.7	57:1	14	35.0	178:1	4	34.0	6:1
S, M	53	19.3	171:1	40	50.2	>10,000:1	24	37.5	> 7,000:1
S, S, M+ / S, M, M+	19	35.4	2,300:1	59	61.7	>10,000:1	6	67.6	59:1
M, M	67	44.4	>10,000:1	30	70.9	>10,000:1	40	83.5	>10,000:1
M, M, M+	31	79.6	>10,000:1	13	75.1	>10,000:1	4	86.5	43:1
M, L	22	78.9	> 7,000:1	9	78.6	>10,000:1	12	88.6	>10,000:1
M, M, L+ / M, L, L+	4	100.0	1,700:1	9	100.0	>10,000:1	.....	.....	.....
L, L	6	71.3	1,100:1	3	85.7	15:1	2	100.0	40:1
Total	274	39.2	>10,000:1	214	53.4	>10,000:1	111	62.5	>10,000:1

[a] S, S = 2 small galls; S, S, S+ = 3 or more small galls; S, M = 1 small and 1 medium gall, etc.

The relation between the number of galls and reduction in yield is shown by comparing losses from 2 galls with losses from 3 or more galls of similar size and location. Although the number of comparisons with 3 or more galls per plant is not large, it is evident that 3 galls caused greater reduction in yield than did 2 galls. On the average the increased reduction from 3 galls per plant is nearly one-third greater than for 2 galls, which indicates that losses from multiple infections are nearly directly proportional to the number of galls per plant. This relationship holds for small and medium galls only, since losses from 2 large galls usually approach 100 per cent.

A comparison of the data in table 1 with those in table 3 clearly shows that multiple galls reduce yield more than do single galls. Single small galls below the ear, chiefly on shoots and suckers (Table 1), reduced the yield 2.0 per cent, while 2 small galls and 3 or more galls below the ear resulted in losses of 12.9 and 19.7 per cent, respectively. The difference in loss between 1 small gall and 2 small ones is 2.1 times the standard error. The same general relationship is shown between single, medium, and large galls and multiple, medium, and large galls; the difference in reduction of yield between 1 and 2 medium galls below the ear is 5.0 times the standard error and between 1 and 2 large galls below the ear 2.7 times the standard error. Similar comparisons between the reduction in yield from 1 and 2 galls above the ear (chiefly main stalk and neck) show that multiple galls were significantly more destructive. A general summary comparing losses from single and multiple galls is given in table 4.

In addition to the losses from single and multiple galls, it was found that among the varieties and crosses grown at University Farm in 1931, 1932, and 1933, .7 per cent of the infected plants was killed as a result of smut infection.

Seventy comparisons with multiple infections were obtained among the inbred lines. In general, the results agreed with those shown for the varieties and crosses.

Ear Characters Associated With Smut

In 1932 and 1933 notes on ear luster were taken, and in 1933 data also were obtained on ear rots between paired comparisons of smut-infected and smut-free plants. No attempt was made to assign a numerical value for either ear luster or ear rots. The better luster and the least ear rot were determined for each paired comparison without knowing which ear was produced by the smut infected plant. If there was no relationship between smut infection and either luster or ear rots, smutted plants should have the best luster in 50 per cent of the cases and the least ear disease in 50 per cent

TABLE 4.—*General summary of reductions in yield from single and multiple smut galls*

Location of smut gall	Size of gall and percentage reduction									
	n.[a]	Small	n.	Small and medium	n.	Medium	n.	Medium and large	n.	Large
Single infections										
Below the ear	165	2	.....	.....	168	17	.....	.....	49	42
Above the ear	173	12	.....	.....	167	33	.....	.....	59	87
Ear	64	2	.....	.....	56	22	.....	.....	112	83
Multiple infections										
All below the ear	62	14	72	24	98	56	26	82	7	85
Above and below the ear	54	23	99	57	43	74	18	89	3	86
All above the ear	23	30	30	44	44	84	.....	.....	2	100

[a] Number of paired comparisons.

of the comparisons. On this basis, statistical treatment of the data could then be made by means of the X^2 test for a 1:1 ratio.

The data given in table 5 are based on those comparisons where the smut-infected and smut-free plant both produced an ear, and includes only those comparisons where distinct differences were shown in ear luster and in ear rots.

TABLE 5.—*Relation of smut infection to ear luster and to infection with ear-rotting organisms*

Location of smut gall	Ear luster			Ear rots		
	No. of comparisons	Percentage of ears with best luster		No. of comparisons	Percentage of ears with most disease	
		Smut-infected	Smut-free		Smut-infected	Smut-free
Single infections						
Below the ear	244	38	62	.....	.....	.....
Above the ear	161	36	64	79	66	34
Ear smut	73	28	72	52	90	10
Multiple infections	218	29	71	106	80	20
Total	701	34	66	237	78	22

The results of these comparisons clearly show that ears borne on smutted plants tend to have a poorer luster than those from their smut-free checks. In 244 comparisons involving single smut galls below the ear, the ear from the smutted plant had the better luster in 38 per cent and the poorer luster in 62 per cent of the cases. The X^2 test based on numbers gave odds greater than 100:1 that the observed frequency deviated from a calculated 1:1 ratio. In all other simple smut infections and in multiple infections the odds were nearly 100:1 to greater than 100:1 that the ear borne on the smut-free plants had the better luster.

The association between ear rots and smut infections in 1933 was even more striking than the relation between smut infection and luster. In 52 comparisons with ear-smutted plants 90 per cent of the smutted ears also had the most ear rots. In the cases involving smut galls below the ear, above the ear, or in multiple infections, the odds based on the X^2 test were over 100:1 that the ear from the smut-free plant had the least ear rot. These results indicate that the plants were apparently predisposed to ear rots following smut infection. Smut infection on the ear apparently affords an accessible avenue for the entrance of ear-rotting organisms, and this may partly account for the relatively large amount of ear rots on smutted ears.

DISCUSSION

The results of these studies may be of considerable importance in determining the losses due to smut. In making a general field survey of losses from corn smut it probably would not be desirable to record the number, size, and position of the smut gall as specifically as is given in the present paper. However, these studies indicate that the average reduction in yield caused by smut infection, irrespective of size, or location of smut galls, was approximately 25 per cent for single galls and about 50 per cent for multiple galls. On the basis of the percentage of plants with multiple and single galls, the average reduction in yield per plant due to smut is estimated to be approximately 30 per cent. Losses also result indirectly from smut infections by lowering the quality of the corn and by increasing the likelihood of mold attack on the ears.

The results of these investigations emphasize the need of breeding for smut resistance in corn. Highly smut-resistant inbred lines of corn have been obtained fairly easily by inbreeding and selection at University Farm. The recombination of these smut resistant lines into desirable hybrid combinations offers the most feasible method of reducing losses from corn smut.

SUMMARY

In 1932 and 1933, at University Farm, St. Paul, Minnesota, over 1800 paired comparisons were made between smut-free and smut-infected corn plants, of which there were hybrids, varieties, and inbred lines. It was shown that the extent of loss in yield of ear corn resulting from smut infection was dependent upon the number, size, and location of the smut galls.

The reduction in yield from multiple galls was significantly greater than that from a single smut gall of similar size and location. On the average, single smut galls reduced the yield about 25 per cent, and multiple galls approximately 50 per cent.

Single or multiple smut galls on the main stalk and neck above the ear were about twice as destructive as smut galls of similar size and number located below the ear, usually on shoots or suckers. Medium-size single or multiple smut galls above the ear frequently caused barren stalks, while large galls above the ear usually resulted in barren stalks.

In 1932 and 1933 approximately .7 per cent of smut-infected plants in all the varieties and crosses grown at University Farm were killed prematurely by smut.

Smut galls on the tip end of the ear were less destructive (loss 23 per cent) than galls of similar size more generally distributed through the ear (loss 43 per cent). Large ear galls usually resulted in barren stalks but small galls on the ear did not reduce yield significantly.

Medium and large smut galls on the tassels caused heavy reduction in yield. Small smut galls on the tassel were not injurious.

Ears from smut-infected plants tended to have a poorer kernel luster than those from smut-free plants, and also were more likely to be infected with ear rots.

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DUSTING AND SPRAYING EXPERIMENTS FOR THE CONTROL OF SUGAR-BEET LEAF SPOT IN SOUTHERN MINNESOTA[1]

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Leaf spot (*Cercospora beticola* Sacc.) of sugar beets occasionally is quite destructive in some areas in southern Minnesota. Hitherto, few experimental data have been obtained regarding direct control of this disease in nonirrigated sections, although it has been effectively controlled by Coons, Stewart, and Larmer[3] and Townsend[4] under conditions of irrigation. The following experiments were made in 1931 and 1932 to determine the relative efficiency of Bordeaux mixture and copper sulphate-lime dust in controlling the disease.

The experiments were made in commercial beet fields near Chaska, Minnesota. Bordeaux mixture (4–4–50) was used for spraying and monohydrated copper sulphate and lime (20–80) for dusting. The Bordeaux mixture was always freshly made from stock solutions. Hydrated lime, such as is sold commercially for finishing plaster, was used for both the liquid and dust fungicides. The liquid applications were made with a 3-gal. compressed-air hand sprayer, equipped with a 3-ft. extension rod and angle nozzle. The dust was applied with a "Hudson" hand duster. Care was taken to insure thorough coverage of all plants at each application. The first applications were made when leaf spot first appeared in the fields (about July 15) and the later applications were made at approximately 2-week intervals.

Plots, 8 rows wide and 100 feet long, were used for all experiments. Each treatment was replicated 5 times. In 1931 the sprayed plots in Field A were arranged in the form of a Latin square; all others were arranged as

[1] The data presented in this paper were obtained in cooperative investigations by the Division of Sugar Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture, and the Division of Plant Pathology and Botany of the Minnesota Agricultural Experiment Station. Paper No. 1240 of the Journal Series of the Minnesota Agricultural Experiment Station.

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[3] Coons, G. H., Dewey Stewart, and F. G. Larmer. The sugar-beet leaf-spot disease and its control by direct measures. U. S. Dept. Agr. Circ. 115. 1930.

[4] Townsend, C. O. Leaf-spot, a disease of the sugar beet. U. S. Dept. Agr. Farmers' Bull. 618. 1922.

randomized blocks. In 1932 the Latin-square arrangement of plots was used for all tests. Four rows were left between the dusted plots to minimize as far as possible the effect of any drifting dust. The 6 inside rows of each plot were harvested. Beets adjacent to obvious skips in the row were discarded and an attempt was made to take beets with normal competition on all sides in determining the yield. All yields are computed on the basis of a 100 per cent stand of beets in the plots. Three 20-beet samples were taken from each plot for determination of percentage sucrose and apparent coefficient of purity. For the former the Sachs-Le Docte method was followed, while the apparent coefficient of purity was determined from the refractometer value and the sucrose percentage of a sample of pulp.

All data were analyzed by the analysis-of-variance method, as outlined by Fisher,[5] the value of z at the 5 per cent point being taken as the criterion for significance of the experiments.

To determine which mean differences were statistically significant, the various means for treatment were compared with those for nontreatment and with each other. In these comparisons the standard errors of the difference between 2 means were determined.[6] To determine the value necessary for significance the appropriate value for t, as given in Fisher's tables, was found and the standard error of the difference multiplied by this figure. This value of t takes into consideration the number of degrees of freedom for error. Individual analyses of variance were made of each test.

Experiments in 1931

The experiments in 1931 were made in fields located near the banks of the Minnesota River, owned by William Schalow and Fred Rauschfleisch. For convenience the fields are designated A and B, respectively. Both fields had a fairly uniform stand and had previously grown sugar beets under short rotations.

In field A leaf spot was scarce on July 16 when the first applications were made, with only 1 to 6 spots per leaf. Two weeks later (July 29), in the untreated portion of the field, the number of spots had increased 4 or 5 times per leaf, and distinct infection foci were evident. By August 13, these foci were more numerous, and the disease spread from these centers to a distance of approximately 4 feet. The prevalence of leaf spot increased rapidly and, by August 27, the condition could be called epidemic. On this date many lower leaves were dead. During the ensuing 2-week period new leaves were produced, and they were in turn destroyed by a second out-

[5] Fisher, R. A. Statistical methods for research workers. Ed. 4, rev. and enl., 307 pp. illus. Oliver and Boyd, Edinburgh and London. 1932.

[6] The standard error of the difference between two means $= \sqrt{\frac{\sigma^2 \times 2}{n}}$.

break of the disease, so that by September 10 only the center whorls of small leaves on the majority of the plants were green.

In field B the disease had not become epidemic by harvest. Although most of the plants in the non-treated plots were infected, there was very little leaf injury.

The results of dusting and spraying in 1931 are summarized in table 1.

Dusting Tests

Analyses of the yields obtained from the dusting tests show no significant differences due to treatment. In field A the disease situation was such that significant differences were to be expected because the disease was controlled. But because of unfortunate circumstances, doubtless related to the stand condition in at least 3 plots, the yields computed from the plots departed widely from the average of the replications. It is to be assumed that, because of poor stand, beets not wholly subjected to normal competition were harvested and that the average weight determined per individual root was too high, thus giving an extremely high tonnage value when this average root weight was multiplied by the number of roots per acre in a perfect stand. When the 2 replications in which these questioned values occur were discarded and the data computed on the basis of the remaining 3 blocks, the results were significant. From these average yields, progressive increases in tonnage with the greater number of applications of dust are obtained. Therefore, under these conditions, it seems fair to state that, although the test as a whole was inconclusive, there are definite trends indicating control of leaf spot and gains in yield. The data on sucrose percentage and apparent coefficient of purity do not indicate significant increases.

In field B the disease caused only slight injury, and significant differences in yield were not obtained. Similarly, the data for sucrose percentages and for apparent coefficients of purity were not significant.

Spraying Tests

Analysis of the yields obtained from the spraying test in field A demonstrates that the variance assignable to treatments was statistically significant for the experiment as a whole. The standard error of the difference of any 2 means in this test was found to be 0.87 tons, and this figure, when multiplied by the appropriate t value (2.179), equals 1.89 tons. This figure is taken as the tonnage difference between any 2 means required to reach the 5 per cent level of significance. By applying this test to the data the average yield, where 2 applications of Bordeaux mixture were made, is not significantly different from that of the control (nonsprayed), but the averages for 3, 4, and 5 applications are significantly higher than from the

TABLE 1.—*Results of dusting and spraying tests with sugar beets in Field A at Chaska, Minnesota, in 1931. The disease was in epidemic amount by August 27*

Kind of treatment	Number of applications	Yield or roots[a]		Sucrose		Apparent coefficient of purity	
		Computed (per acre)	Increase over control	Determined value	Increase or decrease over control	Determined value	Increase or decrease over control
		tons	*tons*	*per cent*	*per cent*	*per cent*	*per cent*
Dust	0	17.89	.....	13.43	.....	86.12	.....
	3	21.07	+3.18	13.71	+0.28	86.10	−0.02
	4	21.07	+3.18	12.91	−0.52	85.88	−0.24
	5	24.30	+6.41	13.04	−0.39	87.58	+1.46
Difference required for significance	.....	.....	[b]		[d]		[d]
Spray	0	19.69	.....	13.94	.....	86.86	.....
	2	20.07	+0.38	14.95	+1.01	87.31	+0.45
	3	21.74	+2.05	14.61	+0.67	88.02	+1.16
	4	22.23	+2.54	14.75	+0.81	88.31	+1.45
	5	22.61	+2.92	14.42	+0.48	88.30	+1.44
Difference required for significance	.....	.....	1.89		[b]		[c]

[a] Yields computed on basis of 100 per cent stand from plot data .

[b] Variance due to error was greater than that due to treatment; therefore, the data are not significant.

[c] Value of observed z did not reach the value of the calculated z at the 5 per cent point.

[d] Data not analyzed, since data for yields were not significant.

control. Three applications were no better than 2, although the difference was suggestively large. Further, 4 and 5 applications proved to be superior to 2 applications, but were not significantly different among themselves. From this experiment, made in fields in which leaf spot was epidemic, it can be concluded that 3, 4, and 5 applications of Bordeaux mixture controlled leaf spot and resulted in significant tonnage increases as compared with the nonsprayed plots. The analyses of sucrose percentages and apparent coefficients of purity demonstrated that they were not significant; therefore, significant differences are not to be expected from the test.

Since leaf spot did not become very prevalent at any time during the growing season in field B, the average yields, sucrose percentages, and apparent coefficients of purity for the various spraying treatments were not significant.

The data obtained in 1931 in the field where leaf spot was epidemic failed, as previously mentioned, to demonstrate that dusting effectively controlled the disease. The results, however, are interpreted as

indicative of a trend in favor of the dusted plots, and they show that favorable results might have been obtained had the stand in the plots been more uniform at the outset. The results with Bordeaux mixture were conclusive in that statistically significant differences were obtained where 3 or more spray applications were made. Because of these indications, experiments of the same type were made in the following season.

EXPERIMENTS IN 1932

In 1932 the dusting and spraying tests were made in fields owned by Arthur Le Mieux and William Ess, both fields consisting of river-bottom land near Chaska, Minnesota. These fields have been designated as A and B, respectively. The stands in both were exceptionally good.

Leaf spot was exceedingly destructive in both fields, causing burning of all lower leaves in the nontreated plots. The plants in the sprayed plots in both fields were well protected from infection and in marked contrast to the nonsprayed plants. The protective value of Bordeaux was evident on visual field inspection, the nonsprayed plants being severely burned, while sprayed plants were green and relatively disease-free. The dusted plants were protected to a certain extent, but apparently not so fully as the sprayed ones. The results are summarized in table 2.

Dusting Tests

Analysis of the yields from the dusted plots in field A show that 2, 4, and 5 applications did not bring about yields significantly higher than those obtained in the nondusted plots. The plots receiving 3 applications appear to yield significantly more than those receiving no dust or 2 applications. This probably is due to chance rather than to superiority of this particular treatment, because the plots receiving 1 and 2 additional applications do not show similar superiority over the control. It should be remarked that the gains in yield over the control, in the case of the plots receiving 4 and 5 applications, are suggestively large and indicate that the dusting was of considerable value. There was a progressive increase in sucrose percentage with an increase in the number of applications of dust. The dusted plots, irrespective of number of applications, were superior in percentage of sucrose to the nondusted plots. Those receiving 4 and 5 applications did not differ significantly in percentage of sucrose, but apparently were significantly better than those with 2 applications. Apparent coefficients of purity were found to be not significantly different; therefore, only gross sugar per acre was calculated. There was increased gross sugar per acre from all dusted plots as compared with the nondusted ones. The gross sugars of plots receiving 3, 4, and 5 applications did not differ statistically among

TABLE 2.—*Results of dusting and spraying tests with sugar beets at Chaska, Minnesota, in 1932*

Kind of treatment	Number of applications	Yield of roots[a]		Sucrose		Apparent coefficient purity		Estimated gross per acre	
		Computed, per acre	Increase over control	Determined value	Increase over control	Determined value	Increase over control	Computed[d] per acre	Increase over control
		Field A: Disease in epidemic amount							
		tons	*tons*	*per cent*	*per cent*	*per cent*	*per cent*	*lbs.*	*lbs.*
Dust	0	16.75	.....	15.54	.....	86.34	.....	5211.2	.....
	2	16.86	0.11	16.20	0.66	88.10	1.76	5472.0	260.8
	3	18.59	1.84	16.42	0.88	87.20	0.86	6112.2	901.0
Difference required for	4	17.78	1.03	16.74	1.20	87.32	0.98	5953.2	742.0
	5	17.82	1.07	17.22	1.68	87.28	0.94	6133.8	922.6
significance	.....	.....	1.17		0.57		[c]		556.8
Spray	0	17.16	.....	15.30	.....	86.42	.....	5248.6	.....
	2	17.21	0.05	16.02	0.72	86.68	0.26	5514.2	265.6
	3	17.84	0.68	16.24	0.94	87.46	1.04	5797.0	548.4
Difference required for	4	18.12	0.96	16.40	1.10	86.44	0.02	5945.2	696.6
	5	18.57	1.41	16.10	0.80	86.68	0.26	5984.2	735.6
significance	.....	.....	0.84		0.33		[c]		265.8
		Field B: Disease in epidemic amount							
Dust	0	27.16	.....	14.58	.....	83.62	.....	7910.4	.....
	2	31.22	4.06	14.94	0.36	83.66	0.04	9329.0	1418.6
	3	32.69	5.53	15.40	0.82	86.16	2.54	10061.8	2151.4
Difference required for	4	31.34	4.18	15.68	1.10	84.94	1.32	9232.0	1321.6
	5	31.40	4.24	15.90	1.32	84.44	0.82	9956.0	2045.6
significance	.....	.....	3.50		0.32		[b]		1394.8
Spray	0	20.39	.....	14.74	.....	82.85	.....	6010.2	.....
	2	21.75	1.36	15.30	0.56	83.52	0.67	6654.8	644.6
	3	22.37	1.98	16.02	1.28	83.74	0.89	7175.2	1165.0
Difference required for	4	23.34	2.95	16.12	1.38	84.16	1.31	7519.8	1509.6
	5	23.81	3.42	16.20	1.46	83.72	0.87	7714.0	1703.8
significance	.....	.....	1.16		0.61		0.71		447.9

[a] Yields computed on basis of 100 per cent stand from plot data.
[b] Variance due to error was greater than that due to treatments; therefore, data are not significant.
[c] Value of observed z did not reach the value of the calculated z at the 5 per cent point.
[d] The estimated gross sugar per acre was obtained by multiplying individual plot yields in pounds per acre by mean percentage sucrose and dividing by 100.

themselves but were significantly greater than those of the plots with only 2 applications.

It is concluded from this test that yield was probably favorably influenced by 3 or more applications of dust and the percentage of sucrose definitely so. The cumulative effect of the favorable influence on these 2 factors is shown by the results obtained for gross sugar per acre, in which 3 or more dust applications are seen to bring about significant increases.

The analysis of the yields of roots from the dusted plots in field B showed that the experiment was significant and that all dust applications were significantly better than the control, but probably about equally effective. The average percentages of sucrose from the dusted plots were significantly higher than from the control and appeared to increase with the number of applications. No significant differences between the apparent coefficients of purity could be demonstrated. The analysis of the data for gross sugar per acre indicates significant increases over the control from 2, 3, 4, and 5 applications, the greatest increase being obtained in the plots receiving 5 applications of copper sulphate-lime dust.

Spring Tests

In field A yields from plots sprayed 2 or 3 times are not significantly higher than from the control, whereas 4 and 5 applications of Bordeaux mixture are about equally effective and are superior to 2 or 3 applications. It appears that the average percentage of sucrose of the sprayed plots, regardless of number of applications, were significantly greater than the average of the nonsprayed plots. The gains over the control increase progressively as the number of applications are increased. The extent of these gains closely approaches the amount required for significance and seems to indicate increased control of leaf spot. The value of z for analysis of the apparent coefficients of purity was below the 5 per cent point, and it is concluded that significant effects attributed to treatment were not obtained. The averages for gross sugar per acre indicate that the increases resulting from 2, 3, 4, and 5 applications were significant when compared with the nonsprayed plots. Four and 5 applications result in significant increases when compared with 2 applications, and 5 applications did not give an average value significantly larger than 4 applications.

The observed value of z for the analysis of the yields from the spraying test in field B greatly exceeded the calculated value of z at the 1 per cent point, so it is concluded that the results are highly significant. It appears that the greatest increase in tonnage resulted from 5 applications of Bordeaux mixture, but the yields of 2, 3, and 4 applications are all significantly greater than those of the nonsprayed plots. The analysis of the percentages of sucrose demonstrates that significant differences exist. All the

TABLE 3.—*Mean yields, percentages of sucrose, apparent coefficients of purity, and estimated gross sugar per acre from 2 dusting tests and 2 spraying tests at Chaska, Minnesota, in 1932*

Kind of treatment	Number of applications	Yield of roots		Sucrose		Apparent coefficient of purity		Estimated gross sugar per acre	
		Mean	Increase over control	Mean	Increase over control	Mean	Increase over control	Mean	Increase over control
		tons	*tons*	*per cent*	*per cent*	*per cent*	*per cent*	*lbs.*	*lbs.*
Dust	0	21.95	.....	15.06	.....	84.98	.....	6560.8	.....
	2	24.04	2.09	15.57	0.51	85.88	0.90	7400.5	839.7
	3	25.64	3.69	15.91	0.85	86.68	1.70	8087.0	1526.2
	4	24.56	2.61	16.21	1.15	86.13	1.15	7592.6	1031.8
	5	24.61	2.66	16.56	1.50	85.86	0.88	8044.9	1484.1
Difference required for significance	.....	.....	1.73	.....	0.31	.....	1.52	.....	704.5
Spray	0	18.77	.....	15.02	.....	84.63	.....	5629.4	.....
	2	19.48	0.71	15.66	0.64	85.10	0.47	6084.5	455.1
	3	20.15	1.38	16.13	1.11	85.60	0.97	6486.1	856.7
	4	20.73	1.96	16.26	1.24	85.30	0.67	6737.5	1108.1
	5	21.19	2.42	16.15	1.13	85.20	0.57	6849.1	1219.7
Difference required for significance	.....	.....	0.67	.....	0.32	.....	0.66	.....	244.3

sprayed plots, except possibly those receiving 2 applications, are significantly better than the nonsprayed plots. Furthermore, 3, 4, and 5 applications are statistically superior to 2, but these applications do not differ among themselves. All sprayed plots except those receiving 2 applications are significantly better than the nonsprayed plots when apparent coefficients of purity are compared, but, apparently, they do not differ significantly among themselves. A consideration of gross sugar per acre indicates that the sprayed plots are significantly better than the nonsprayed ones. Three, 4, and 5 applications resulted in significant increases when compared with 2 applications. Three and 4 applications were found not to differ significantly, but 5 applications are apparently better than 3. The results of spraying in this field, as a whole, indicate that 4 or 5 applications result in outstandingly higher increases.

Summary of the 1932 Tests

Since the experiments in both fields in 1932 were arranged in a like manner and leaf spot was epidemic in both fields, the data have been averaged and are presented in table 3.

The mean yields from the 2 dusting tests in both fields demonstrate that 2, 3, 4, or 5 applications of copper-lime dust are significantly superior to no treatment. The percentages of sucrose for all applications are significantly higher than the nontreated plots. There is a progressive increase in percentage of sucrose with increase in number of applications. A comparison of the apparent coefficients of purity show that an increase, when compared with the controls, was obtained with only 3 applications of dust. It is thought that this probably is due to chance, since plots receiving 4 and 5 applications are not significantly superior to the nondusted ones. The yield of gross sugar per acre was increased significantly over the controls by all applications.

The results of mean yields from the 2 spraying tests show that all applications give significantly greater yields than those from the nonsprayed plots. Three applications are significantly better than 2, but not superior to 4. Five applications of spray give significantly higher yields than either 2 or 3, but are not superior to 4, although the difference closely approaches significance. Percentages of sucrose for all applications of spray are significantly better than the control and 3, 4, and 5 applications are distinctly superior to 2, but they are not significantly different one from the other. Comparison of mean differences of the apparent coefficients of purity shows that no significant increase results from 2 applications of Bordeaux mixture, whereas 3 and 4 applications are both superior to the control; 5 applications closely approach significance. The values of the apparent coefficients of purity of these 3 applications, however, are not significantly dif-

ferent one from another. Progressively significant increases in the yield of gross sugar per acre were obtained with 2 and 3 applications when compared with the control. Four and 5 applications are not significantly different, but are decidedly better than the control and 2 or 3 applications.

From the data in table 3, the percentage increase in yield for 5 applications of dust over the control is 12.12 per cent, whereas for 5 applications of spray it is 12.89 per cent. Thus it appears that the 2 treatments probably were equally effective in controlling leaf spot.

SUMMARY

This report presents the results of 2 season's experiments on the efficiency of copper sulphate-lime dust (20–80) and Bordeaux mixture (4–4–50) for the control of Cercospora leaf spot in the vicinity of Chaska, Minnesota.

In 3 tests, made under epidemic conditions of the disease, significant increased yields when compared with the controls, were obtained in all spraying tests, whereas significant increases from dusted plots were obtained in only 2 tests; the third test, however, approached significance.

From field observation, the dusted plots had a somewhat lower measure of leaf-spot control than the sprayed plots, but a comparison of only statistically significant data from dusting and spraying tests indicates that the percentage increase in yield due to dusting and spraying is nearly identical.

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ANTAGONISTIC ACTION OF TRICHODERMA ON RHIZOCTONIA AND OTHER SOIL FUNGI[1]

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It has been demonstrated by a number of workers, that antagonistic relationships exist between many microörganisms, and in a few cases the phenomenon has been observed in plant pathogens. Sanford and Broadfoot (5) isolated 6 species of fungi and 15 species of bacteria that were able markedly to suppress the pathogenicity of *Ophiobolus graminis*, and Vasudeva (6) found that *Botrytis allii* inhibited decay in apples caused by *Monilia fructigena*. Weindling (7) recently reported that *Trichoderma lignorum* is parasitic on Rhizoctonia and several other fungi and suggests the possibility that this fungus might be used to control certain soil-borne pathogens. Brown (2) has found also that the presence of Trichoderma may cause a reduction in the amount of Texas root rot induced by *Phymatotrichum omnivorum*.

During the course of a series of greenhouse and laboratory studies on seed decay and seedling damping off caused by Rhizoctonia and Pythium, a number of tests were conducted that corroborate the observations made by Weindling concerning the antagonism between Trichoderma and Rhizoctonia and that have thrown some light on the probable nature of the antagonism in this case. The results of these greenhouse and laboratory tests are briefly presented.

GREENHOUSE STUDIES

In the first test an unsterilized potting soil was placed into half-gallon glazed earthenware pots, 24 of which were inoculated heavily with a pure culture of Rhizoctonia and 24 with Pythium. Twelve pots from each of these series were given an additional heavy inoculation with a pure culture of *Trichoderma lignorum* grown on sterilized whole oats.

Three days after inoculation half of the pots were planted with cucumber and half with garden pea seeds and allowed to remain in a warm, moist greenhouse where conditions were favorable for seed decay and damping off. In this test, the results of which are presented in the first part of table 1, it will be seen that, in the case of cucumber, the amount of seed decay and damping off was appreciably reduced in the series inoculated with Trichoderma, thus indicating that the presence of this fungus had in some way interfered with the parasitic action of the Pythium and Rhizoctonia. In

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Plant Pathology.

the pots planted to peas the results were less marked, the Rhizoctonia series again showing beneficial effects from the Trichoderma, while the Pythium series gave negative results.

After removing the seedlings in the above test the pots were replanted to the same crop without further soil treatment in order to determine if the effect of the Trichoderma would persist for any length of time. At the

TABLE 1.—*Percentage of normal seedlings obtained from seeds planted in soil inoculated with Pythium or Rhizoctonia used alone or in combination with Trichoderma. Counts 14 days after planting.*

Seed	Inoculum			
	Pythium and Trichoderma	Pythium alone	Rhizoctonia and Trichoderma	Rhizoctonia alone
		First planting		
Cucumbers (90 seeds)	36.7	7.8	33.3	17.8
Peas (60 seeds)	34.0	40.0	53.8	41.7
		Second planting		
Cucumbers (90 seeds)	72.0	49.9	81.1	37.8
Peas (60 seeds)	55.0	30.0	55.0	31.7

time the second planting was made a month had elapsed since the inoculations. The results from this test, as shown in the second part of table 1, again showed a marked reduction of seed decay and damping off of both cucumbers and peas in the soils containing Trichoderma.

Another and similar test was conducted with soil previously sterilized with formaldehyde. After thorough aeration, the soil was placed into 72 pots arranged into 6 series of 12 pots each and inoculated as follows: Series 1—Pythium alone; Series 2—Rhizoctonia alone; Series 3—Trichoderma alone; Series 4—Pythium and Trichoderma combined; Series 5—Rhizoctonia and Trichoderma combined; and Series 6—Sterilized oats alone. The fungi in each case were grown on sterilized whole oats and both fungus and substratum were used as the inoculum.

Three days after inoculation the pots were planted to cucumbers or peas, 6 pots for each treatment, as in the former tests. The results, presented in table 2, again show a marked increase in the number of healthy seedings obtained where Trichoderma was used with Pythium or Rhizoctonia as compared with the series where the pathogens were used alone. As in the first tests, cucumbers again showed the greatest benefits from the presence of the Trichoderma.

The results of these greenhouse tests coincide with those obtained by Weindling (7) relative to the antagonistic action of Trichoderma on Rhizoc-

tonia and Pythium. They show clearly that the Trichoderma interferes with the normal activities of these pathogens, but they give no suggestions as to how this antagonism is brought about.

TABLE 2.—*Per cent of normal seedlings obtained from seed planted in Formalin-sterilized soil, subsequently inoculated with Pythium and Rhizoctonia, used alone or in combination with Trichoderma. Counts 14 days after planting*

Seed	Inoculum					
	Sterile oats	Trichoderma alone	Pythium and Trichoderma	Pythium alone	Rhizoctonia and Trichoderma	Rhizoctonia alone
Cucumber (90 seeds)	80.0	80.0	87.8	31.1	87.8	33.3
Peas (60 seeds)	46.7	40.0	53.3	23.3	61.7	36.7

PHYSIOLOGICAL STUDIES

After it had been found that Trichoderma seems to inhibit the parasitic activities of Rhizoctonia and Pythium in pot tests under greenhouse conditions, a number of laboratory experiments were conducted to further study the nature of this antagonistic action.

Porter (4), Broadfoot (1), Sanford and Broadfoot (5) and Fawcett (3), working on the association of certain organisms, have suggested that the inhibitions produced are caused by some toxic or enzymatic substance elaborated during the growth of the organism; and Weindling (7) states that *Trichoderma lignorum* parasitizes *Rhizoctonia solani* by means of some diffusible toxic or enzymatic substance. Very few statements are made by these workers, however, that give any indication as to the stability or other physiological properties of the toxic products formed. The following laboratory experiments, conducted with *Trichoderma lignorum*[2] and a strain of *Rhizoctonia solani* isolated from Lima beans, give some information along this line.

Preliminary Tests.—In a preliminary study, dextrose-agar plates were inoculated on opposite sides with Trichoderma and Rhizoctonia to observe whether an antagonistic zone would be produced between the 2 colonies. As the growth spread in these plates, it was noticed that the Rhizoctonia stopped growing when the Trichoderma mycelium approached within a few millimeters; the Trichoderma, on the contrary, spread over the Rhizoctonia mycelium as though the latter were not present. Such antagonism between

[2] This fungus has the general characteristics of *T. lignorum*, but the species has not been identified with certainty.

fungi grown in adjacent colonies in solid media has been observed by many and is of interest here only in that it demonstrates that the Trichoderma used in these experiments is antagonistic to the Rhizoctonia when grown in artificial media. Another preliminary experiment was conducted with flasks containing 100 cc. of dextrose broth.[3] These were inoculated with Trichoderma and Rhizoctonia alone or with the 2 organisms together to see which organism would dominate the other. After 4 days' incubation, the pure Rhizoctonia and the pure Trichoderma cultures showed excellent growth. Where Rhizoctonia and Trichoderma were together in the same culture, it was clearly evident that the latter had completely dominated. This was shown by lack of browning of the mycelium and of the nutrient solution, which was characteristic of all cultures containing Rhizoctonia alone. The mixed cultures appeared to be similar to the pure cultures of Trichoderma in every respect. Both of these preliminary experiments show that there is some inhibiting action of the Trichoderma on the growth of Rhizoctonia.

Toxicity of Filtrate from Trichoderma Culture. Several experiments were conducted to determine whether the filtrate from a liquid Trichoderma culture would inhibit the growth of Rhizoctonia or whether the inhibition occurs only in close proximity to the living Trichoderma cells. The 2 organisms were grown separately in dextrose broth for a period of 5 days, after which the luxuriant fungous mats were filtered off and the filtrate sterilized by passage through a Seizts filter. These filtrates were transferred to sterile flasks and half of each series inoculated with Rhizoctonia and the other half with Trichoderma. Control flasks containing fresh nutrient media also were inoculated with each organism. The results were very significant. In every case where the Rhizoctonia was inoculated into the filtrate from the Trichoderma cultures, there was no growth, (Fig. 1, 1). In all other cases the organisms grew as well as in the control flasks, showing definitely that there was no lack of nutrients in the filtrates, (Fig. 1, 2). It was also evident that Rhizoctonia produced no toxic product that was injurious to either Rhizoctonia or Trichoderma. (Fig. 1, 3, 4.) This same experiment was conducted 3 times, always with the same results. This is conclusive evidence that an active principle inhibitory to the growth of Rhizoctonia is elaborated into the medium by the Trichoderma and that the immediate presence of the organism is not necessary to cause the inhibition.

Destruction of Toxic Principle by Heat.—A test was conducted to determine whether the active principle produced by the Trichoderma is destroyed by heat sterilization. Filtrates from Trichoderma and Rhizoctonia cultures were prepared as before. Half of each series was sterilized by

[3] Medium used: Dextrose, 40 g.; asparagin, 2 g.; K_2HPO_4, 0.5 g.; distilled water, 1,000 cc.

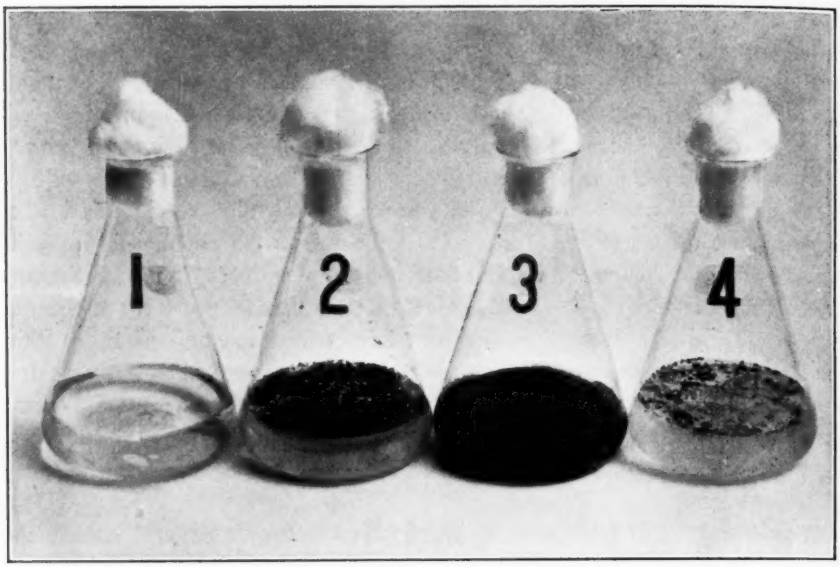

FIG. 1. Culture No. 1—Rhizoctonia inoculated into filtrate from Trichoderma.
" No. 2—Trichoderma " " " " "
" No. 3—Rhizoctonia " " " " Rhizoctonia.
" No. 4—Trichoderma " " " " "

passing through a Seizts filter and the other half by autoclaving for 20 minutes at 15 pounds' pressure. These 4 series were again divided, half of each lot being reinoculated with the organism grown on the media before sterilization and the other half being inoculated with the other organism.

The filter-sterilized series gave the same results as in the first test, that is, no growth occurred in the Trichoderma filtrate inoculated with Rhizoctonia, while normal growth was obtained on all other cultures. In the autoclaved series all cultures grew normally, indicating that the active principle was destroyed by heat sterilization.

In order to determine the thermo-stability of the toxic principle elaborated by the Trichoderma, a series of filtrates from 5-day-old Trichoderma cultures were sterilized with the Seizts filter and then subjected to various heat treatments. Large test tubes, each containing 25 cc. of the filtrate, were held for a period of 10 minutes at temperatures ranging from 50° to 120° C. at 10-degree intervals. After the heat treatment, they were immediately cooled to room temperature and 4 tubes of each series were inoculated with Rhizoctonia and 1 with Trichoderma. All of the Trichoderma cultures showed growth on the second day and grew normally thereafter. The growth of Rhizoctonia, on the other hand, varied according to the heat treatments received by the filtrates. The results are shown in table 3. It

will be noted that no growth of Rhizoctonia occurred in filtrates heated at a temperature lower than 80° C. Heating at 80° for 10 minutes reduced the toxicity of the filtrate appreciably, while heating at 90° left the filtrate only very slightly toxic. In filtrates heated at 100° C. or above, the toxic principle was apparently completely destroyed, as was shown by the fact that Rhizoctonia grew as well in the heated filtrates as in the fresh, unused medium.

The gradual decrease in the toxicity shown by filtrates heated at 70, 80, 90, and 100° C., respectively, may indicate that the toxic principle in the filtrate consists of several products differing in their thermal relationships, as was found by White (8) in filtrates from *Fusarium lycopersici* toxic to tomato plants.

TABLE 3.—*The destruction of the toxic principle in Trichoderma filtrates by means of heat. Growth of Rhizoctonia in filtrates that had been exposed for 10 minutes to various temperatures*

Temperature C.°	Growth	Days delay in growth start	Remarks
Non-heated	None	Infinite	Inoculum died
50	"	"	" "
60	"	"	" "
70	"	"	" "
80	Very slow	5	Mycelium abnormal
90	Slow	1	Finally recovered
100	Luxuriant	0	Growth as good as in fresh nutrient
110	"	0	Growth as good as in fresh nutrient
120	"	0	Growth as good as in fresh nutrient

Destruction of the Active Principle by Aging and Oxidation.—An experiment was undertaken to determine how long the toxic principle would remain active in the Trichoderma filtrate. In this experiment a series of filter-sterilized Trichoderma filtrates were set up in cotton-plugged tubes and inoculated with Rhizoctonia after intervals of 1, 3, 5, 10 and 20 days. No growth appeared in tubes inoculated on the 1st, 3rd, and 5th days. A retarded growth occurred in tubes inoculated on the 10th day and normal growth in those inoculated after 20 days.

Other tubes of sterile Trichoderma filtrates, that had been inoculated with Rhizoctonia 20 days previously and in which no growth had occurred during the time, were reinoculated with Rhizoctonia. These reinoculations

grew normally, indicating that the inoculum first used was killed by the toxic filtrate and not merely held in a state of dormancy. The results of these tests show that the toxic principle in the fresh Trichoderma filtrate is lethal to Rhizoctonia mycelium and that the toxic properties are destroyed in some way when the filtrate is allowed to stand for 10 days or more in cotton-plugged glass containers.

To determine whether the inactivation of the filtrate by aging might be due to oxidation, 2 lots of tubes containing active filtrate were subjected to oxidation by bubbling oxygen through them for 5 and 10 minutes, respectively. These treatments apparently entirely inactivated the filtrates, as was shown by the fact that all tubes so treated gave normal growth of Rhizoctonia, while no growth appeared in control tubes of Trichoderma filtrate not treated with oxygen. The results of this experiment show that the toxic principle in the Trichoderma filtrate is readily destroyed by oxidation and suggest that the lack of toxicity of filtrates that have stood for 10 days or longer may be due to a slow oxidation of the toxic products in the filtrate.

Potency of the Toxic Principle.—In order to determine the approximate strength of the active principle of the Trichoderma filtrate the filter-sterilized medium was diluted with various amounts of water, autoclaved filtrate, or fresh unused culture media, respectively. Five tubes containing 25 cc. of liquid were used for each dilution. Four of these were inoculated with Rhizoctonia and one with Trichoderma. The growth in all of the Trichoderma tubes was normal, regardless of the concentration of the filtrate used, showing, as in former tests, that the Trichoderma filtrate contains no products injurious to the Trichoderma itself. Rhizoctonia, on the other hand, failed to grow unless the filtrates were diluted to a considerable

TABLE 4.—*Potency of toxic principle in filtrate from Trichoderma. Growth of Rhizoctonia in toxic filtrate diluted with various proportions of water, autoclaved filtrate, or fresh nutrient medium*

Ratio toxic filtrate to diluent. Parts by volume		Growth of Rhizoctonia in filtrate diluted with the various diluents		
Toxic filtrate	Diluent	Water as diluent	Autoclaved filtrate	Fresh nutrient medium
5	0	none	none	none
4	1	"	"	"
3	2	"	"	"
2	3	retarded	severely retarded	retarded
1	4	vigorous	slightly retarded	vigorous
0	5	.....	vigorous	"

extent. The dilutions used and the results obtained with the Rhizoctonia cultures are given in table 4.

It will be noted from table 4 that no growth of Rhizoctonia occurred in the Trichoderma filtrates when used full strength or when diluted with approximately equal parts of either water, or deactivated (autoclaved) filtrate or fresh sterile medium. When 2 parts of the filtrate were diluted with 3 parts of the diluent, growth was delayed for several days and in one series was very slow and abnormal for several weeks. Slight retardation in growth also was observed in one series where 1 part of the active filtrate was diluted with 4 parts of the autoclaved filtrate.

It will be noted that both water and fresh nutrient gave similar results when used as diluents. When the Trichoderma filtrate, deactivated by autoclaving, was used, however, there was slightly more retardation in growth. This would suggest that the autoclaving had not completely destroyed all of the toxic materials in the filtrate, despite the fact that apparently normal growth was obtained when the autoclaved filtrate was used directly.

The above dilution experiment was repeated, using water and autoclaved filtrate as diluents, with essentially the same results.

Effect of Toxic Principle on Other Fungi.—In a number of the laboratory experiments discussed above, a few cultures were included in which *Pythium de Baryanum* was inoculated into the Trichoderma filtrate. In every case Pythium proved to be just as sensitive as Rhizoctonia to the toxic products. A few preliminary tests also were conducted with other soil-borne pathogenic fungi but the results from these are not conclusive. It seemed evident, however, that the toxic principle in the Trichoderma filtrate was not equally toxic to all fungi. Some of the fungi grew just as luxuriantly in the active Trichoderma filtrate as in the fresh nutrient.

SUMMARY

Seed decay and damping off of cucumbers induced by Rhizoctonia and Pythium were appreciably reduced by inoculating the soil heavily with a species of Trichoderma. Garden peas showed a similar but less marked response.

Laboratory tests indicate that the Trichoderma may have an antagonistic effect on the growth of Rhizoctonia and Pythium.

The presence of an active principle, toxic or lethal to Rhizoctonia, was demonstrated in a liquid medium in which Trichoderma had grown for 5 days.

The filtrate from a 5-day-old Trichoderma culture was lethal to Rhizoctonia when used full strength or when diluted 40 per cent or less with nontoxic diluents.

The toxic principle in the filtrate was completely inactivated or destroyed by heating for 10 minutes at a temperature of 100° C. The toxicity of the filtrate was greatly reduced by a 10-minute exposure at 90° C. and slightly reduced at 80° C.

The potency of the toxic principle was destroyed by bubbling oxygen through the filtrate for a period of 5 minutes, or by allowing the sterile filtrate to stand in cotton-plugged test tubes at room temperature for a period of 20 days.

Rhizoctonia failed to grow in a freshly prepared filter-sterilized filtrate from a 5-day-old Trichoderma culture, but grew luxuriantly in a similar filtrate after the toxic principle had been inactivated or destroyed by the treatment mentioned above; viz., dilution, heat, exposure to oxygen, exposure to air.

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OBSERVATIONS ON TUBERCULINA MAXIMA, A PARASITE OF CRONARTIUM RIBICOLA

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INTRODUCTION

The purple mold, *Tuberculina maxima* Rostrup (*T. persicina*), has been known as a parasite of *Cronartium ribicola* Fischer for many years in European regions where blister rust is common. Saccardo (9) described this interesting genus in 1880, and in his more recent works (10, 11) he has described the species: *T. persicina, T. maxima* and *T. vinosa* Sacc. Gobi (1), studying a fungus that he called *Tuberculariа persicina* Ditm., left the identity of the organism somewhat in doubt. Later, Morini (5), working with *T. vinosa,* made a study of the spores and their process of germination. The latter's work did not agree with Gobi's findings, and there is, therefore, some difficulty in knowing what organisms were used in these early studies.

The species *Tuberculariа maxima* was described by Rostrup (8) in 1890 as attacking *Cronartium ribicola* (*Puccinia klebahni*) on *Pinus strobus* in Denmark and Germany. Rostrup separates *T. maxima* from *T. persicina* on slight differences in spore measurements and in color, but the present tendency is to treat *T. persicina* as a synonym.

Tubeuf (14), in 1902, records his observations made on *Tuberculina maxima* and finds the fungus always in direct association with rusts. The spores were found to germinate readily in water, sugar solutions, and acidified gelatin to which fruit juices had been added. Tubeuf states that the spores of this fungus are not produced catenulate in a jelly-like mass but are produced singly by abstriction from the ends of the sporogenous hyphae, and that the air currents are the principal means of dissemination. Tubeuf (15, 16, 17, 18) has been the principal champion in Germany of the use of *T. maxima* as an agency in the control of the white-pine blister rust. Observations and experiments were continued by him until his recent paper (19) appeared in 1930 reporting the successful use of the parasite in greatly reducing or entirely inhibiting the production of aeciospores of the rust in certain regions of Germany.

Lechmere (3) in 1914 gave additional details regarding the life history of the purple mold and concluded that it restricted its attack to the pycnial and aecial structures entirely and did not penetrate the pine host tissues, nor did it attack or destroy the rust mycelium. Spaulding (12), in 1929, and Rohmeder (7), in 1931, report a marked reduction in aeciospore pro-

duction in several regions, including England, Denmark, Belgium, and Germany. Rohmeder's reports cover several areas in the Swabian forest regions.

In this country the first note of the occurrence of *Tuberculina maxima* was made by Weir and Hubert (20) in 1917, recording the presence of this parasite on the native pine rusts in Montana. Since that time the purple mold has been more commonly observed on native rusts throughout the Western United States and Canada. Mielke (4) and others have collected it in fairly large quantities in British Columbia, and, in 1932, R. K. Pierson collected a purple fungus on the rust cankers that he was investigating on the Newman Lake study plots in Washington. This fungus, upon examination, proved to be *T. maxima*. H. N. Putnam, in October, 1932, found a vigorous development of the fungus on the aecial and pycnial pustules of cankers developing upon the test trees planted on the Cheekye experimental plots in British Columbia. He states that the purple mold completely in-

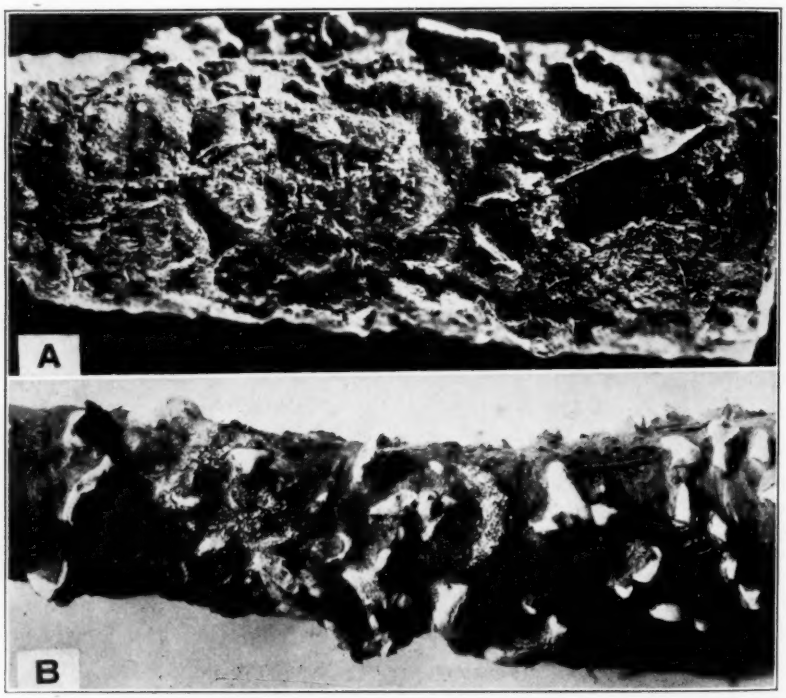

FIG. 1. A. Aecial scars of *Cronartium ribicola* on *Pinus monticola* overgrown by the rust parasite, *Tuberculina maxima*. Note the moldy appearance and absence of peridia. Slightly enlarged. B. Noninfected aecial blisters of *C. ribicola* on *P. monticola*, showing clear-cut outlines and the absence of moldy growth. Normal condition of rust canker.

hibited the aecial development on the parasitized cankers, and that it was found attacking about 1 per cent of the total number of fruiting or aecia-bearing cankers (Fig. 1).

The following is a summary, exclusive of herbaria, of the rust hosts for the purple mold in this country, including the years 1917–1932.

SUMMARY OF RUST HOSTS FOR *T. maxima* IN THE UNITED STATES, THE COLLECTORS AND LOCALITY OF THE COLLECTION

1917.	*Cronartium coleosporioides*	O & I	Weir & Hubert, Montana
1917.	*Cronartium pyriforme*	O & I	Weir & Hubert, Montana
1917.	*Cronartium cerebrum*	O & I	Weir & Hubert, Michigan
1930–31.	*Cronartium ribicola*	O & I	Mielke, *et al.*, B. C.
1932.	*Cronartium ribicola*	O & I	Pierson, Washington
1932.	*Cronartium ribicola*	O & I	Putnam, B. C.
1917.	*Uredinopsis mirabilis*	I	Hubert, Idaho
1932.	*Cronartium coleosporioides*	O & I	Hubert, Idaho
1932.	*Cronartium coleosporioides*	O & I	Chapman, Montana

LIFE HISTORY OF TUBERCULINA MAXIMA

Tuberculina maxima is classified under the Fungi Imperfecti and but one spore stage is known. The conidiospores are large, globose and lilac-colored. A set of 25 spore measurements made of the purple mold developing upon *Cronartium pyriforme* shows the following record: (25) 8.4–13.2 × 12.1–20.9 μ. Av. 10.2 × 12.4 μ. Standard (10 × 18 μ.) The spore measurements given by Saccardo for *T. persicina* are somewhat smaller (7–10 μ) than those usually recorded for *T. maxima*, and Plowright gives 7–14 μ as the range in diameter. Rostrup (8) records the color of *T. persicina* spores as clear violet and states that in contrast to this the spores of *T. maxima* are blue to violet in color. Facts concerning the life history of the purple mold are meager, and it is supposed to attack the spores of its host but not the hyphae. It is not known to attack the bark or wood tissues of the white pines. Observations made in 1917 and since that date, on the habits of the purple mold in attacking *C. pyriforme*, indicate that the fungus develops frequently upon the pycnial stroma beneath the pine epidermis or along the bark cracks. It also sporulates through the pycnial scar openings in the bark tissue. Similar observations have been made regarding its method of developing on *C. ribicola* lesions.

Little seems to be known regarding the overwintering of *Tuberculina maxima*, although some thick-walled spore form would be indicated if it is true that the mycelium develops only in the spore masses and pycnial fluid of the rust fungus. However, it is not improbable that the mycelium may overwinter in the pycnial layer beneath the bark of the host, since examinations made under the microscope of sections cut through pycnial and aecial

areas infected with the purple mold show its mycelium penetrating into the sporogenous layer of the rust fungus. It is reported attacking the uredial stage of rusts but so far it has not been observed on this stage of the blister rust in the Inland Empire or Pacific Northwest regions.

The conidiospores are produced singly from the ends of sporogenous hyphae and form a dense, moldy to powdery mass of purple spores on the subepidermal pycnial stroma, on dried pycnial exudates, pycnial scars and aecial peridia.

The fungus, both the British Columbia strain and the strain obtained from Dr. Tubeuf in Germany, has been tried out on malt agar and a number of other cultural media, but, up to 1933, no successful medium had been found. A study to determine the best artificial media for the rapid development of a large number of spores under laboratory conditions was undertaken by D. J. Stouffer (13) in 1931, but without success. It has, however, been cultured by placing upon malt-agar slants small strips of infected bark bearing the purple mold; but the cultures did not develop independently upon malt agar and soon died. In June, 1933, a pure culture of *Tuberculina maxima*, growing on cherry agar, was received from the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. This culture was successfully transferred to a malt agar, the agar of which was obtained from Sweden. Sporulation on both agars has taken place, but the spore production, as well as the mycelial development, has been slow. No attempts have yet been made to inoculate blister rust cankers with this strain.

The parasite, more aptly termed the purple mold of rusts, sometimes called the lilac fungus, has been successfully employed in the laboratory as inoculum upon the pycnial stage of the white pine-blister rust, developing on cankers obtained from the Ruby Creek area near Bovill, Idaho.

The spores of this fungus have been repeatedly tested in the laboratory and, in most cases, successfully germinated (Fig. 2) in various solutions by a number of workers. Laboratory studies show that the spores are constricted from the ends of sporogenous hyphae, as reported by other workers. The spores germinate within 12 hours after placing in the culture solutions and produce short, broad, unbranched, hyalin germ tubes. On the third day, in 1 per cent glucose, germination had a tendency to slow up. Branching of the germ tube began to take place on the third and fourth days and by the end of the seventh day the hyphae had attained lengths ranging from 100 to 110 μ. The mycelium is characteristically slow in growth.

RECENT FIELD STUDIES

In 1931 the writer became interested in the fungi found attacking the white-pine blister rust and, on March 30, he wrote to Dr. Tubeuf in Germany for a supply of spore material collected from the *Tuberculina maxima*

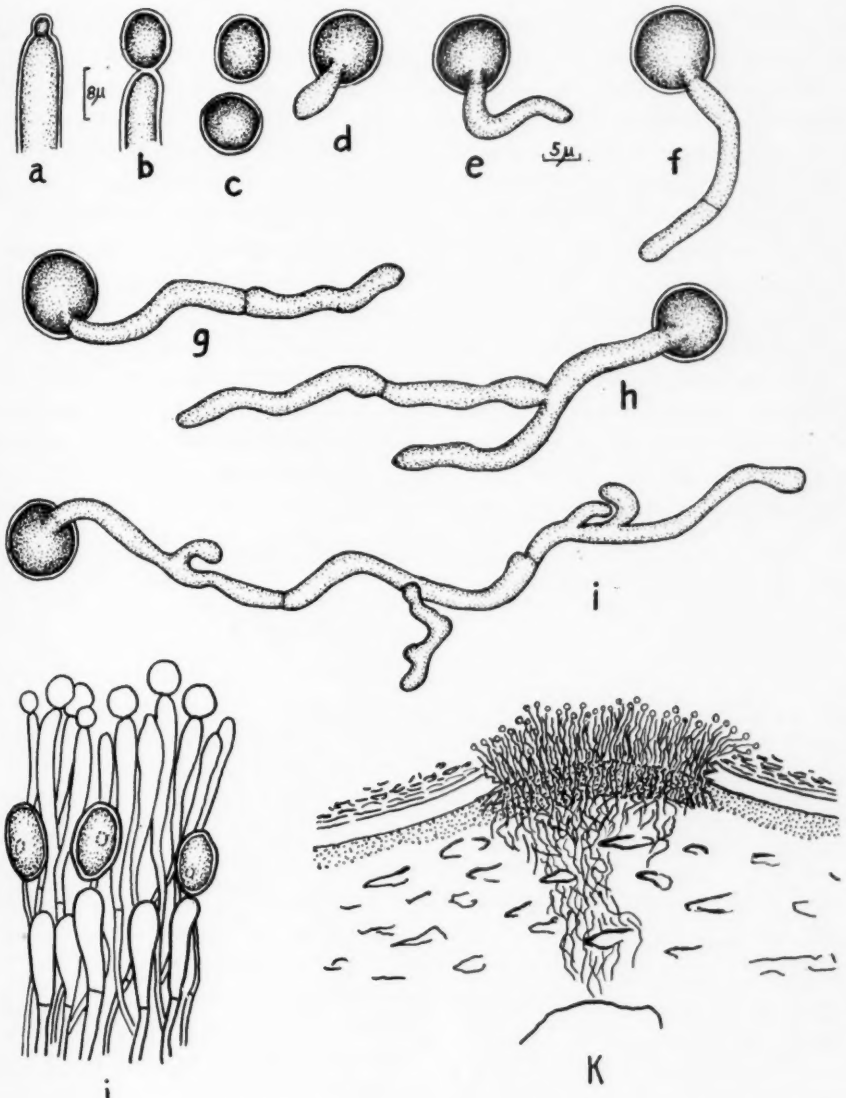

FIG. 2. Camera-lucida drawings showing details in the germination of spores of the purple mold, *Tuberculina maxima*. Germinated at room temperature in hanging drops of 1 per cent glucose solution. a. The end of a sporogenous hypha with a spore in the process of development. b. Nearly mature sport almost severed from the supporting hypha. c. Mature spore (*T. maxima* on *C. coleosporioides*). d, e and f. Germinating spores (*T. maxima* on *C. ribicola*). g. Branched hypha with septa. h. Hypha with swollen segments (*T. maxima* on *C. ribicola*). After 4 days. i. After 7 days. j. Semi-diagrammatical sketch showing the sporogenous layer of the purple mold developing at the surface of the blister rust aecial sporogenous layer. The larger spores are aeciospores. k. Diagrammatical sketch showing the sporogenous layer of the pycnial stage (stippled area) of *C. ribicola* and above it breaking through the bark opening the sporogenous layer of *T. maxima*. Below are shown the hyphal strands of the purple mold extending for a considerable depth into the canker tissue. The blister-rust mycelium is not shown.

infections on blister rust common in that country. Dr. Tubeuf very generously sent a large supply of the spores, which was received in August of the same year. Due to the lateness of the season and to a considerable extent to the extreme dryness of the season, but few field inoculations were made; 12 at Cameron Creek near Elk River, Idaho, and about 10 at Three Bear Creek near the same town. Of the 12 cankers that were inoculated at Cameron Creek 6 were collected in June of the following summer and all of these were dead when cut from the trees. Since a number of the uninoculated cankers also died during this period and subsequent to it, no reliance could be placed upon the results. The inoculations made at Three Bear have produced no results to date.

In 1931 and 1932, collections of the purple mold made at various points in British Columbia were received from J. L. Mielke and T. S. Buchanan of the Division of Forest Pathology and stored in the refrigerator as soon as received. Not all of the last collection, received in August, 1932, was used for field inoculations, but a portion was stored in the refrigerator for

TABLE 1.—*Showing field inoculations made with Tuberculina maxima on blister rust lesions of white pine in Idaho*

Locality	No. of inoculations	Source of purple mold and date collected	Date of inoculations	Number of lesions showing			Results
				Fresh pycnia	1931 aecia	1932 aecia	
Elk River, Idaho Cameron Creek	12	Germany, July, 1931 (Tubeuf)	August, 1931	0	12 old	.....	Negative June 4, 1932 6 cankers dead or dying
Elk River, Idaho Three Bear Creek	10	"	August, 1931	8	10 old	.....	Negative July, 1933
Clarkia, Idaho Middle Fork St. Maries	24 #1–24	Owl Creek, B. C., July 25, 1932	August 3, 1932	21	.....	3	Positive in two cases only. Aug., 1933
Coeur d'Alêne Burnt Cabin Creek	9 #25–33	"	August 27, 1932	9	.....	5	Negative 1933
Oxford R. Sta. Elk Creek, Clearwater, N. F.	4 #34–37	"	August 8, 1932	4	.....	4	Negative July, 1933
Weitas R. Sta. Mouth of Hemlock Creek	13 #38–50	Owl Creek, B. C., August, 24, 1932	Sept. 9, 1932	13	.....	2	Positive in 3 cases only. July, 1933

subsequent study. These collections, fresh from the field and consisting of small thin strips of bark bearing the purple mold and its spores, were used during the summer of 1932 to inoculate a large number of young and old cankers in widely separated regions in northern Idaho. Table 1 gives a summary of the field inoculations made by tieing with heavy thread small pieces of purple molded bark, bark face down, against fresh pycnial exudations or fresh aecial eruptions. All inoculations were labeled with numbered wooden tags fastened to the twigs and branches by means of the fine copper wire attached to them. In some cases the purple spore masses were gently rubbed over the entire surface of the canker.

As the data in Table 1 plainly show, the results of field inoculations produce negative results with the exception of two lesions in the Clarkia area and 3 lesions in the Hemlock Creek area. There are, unquestionably, many factors that are responsible for the weak foothold that the purple mold secured as a result of the 72 inoculations made at various points in Idaho. So little is known regarding the life history of this organism that only a guess can be made as to the most favorable season for applying the spores, the correct stage in the development of the blister rust fungus when these spores are most effective, and the weather conditions most favorable for spore germination and infection. To determine if the spores of the purple mold material were viable, periodic germination tests of the material stored in the refrigerator were made. The spores collected on September, 1932, and stored immediately upon receipt showed good germination, between 8 and 10 per cent on Feb. 4, 1933. On March 17 of the same year, a germination of about 5 per cent was recorded; one or two spores showed germ tubes on April 20 and, when tested on May 20, no germination took place. The spores, under cold-storage conditions at least, are viable for a period of 7 months following their collection in the field.

That the fungus is capable of establishing itself and spreading is evidenced by the infections reported on blister rust cankers at Newman Lake in 1932 and the spread of this infection to neighboring cankers in 1933 and 1934. The spread has been noted also in the British Columbia areas where the purple mold has become established. The difficulty experienced in growing this fungus on artificial media in the laboratory may indicate that it requires rather exacting environmental conditions in nature for its inception and development. We may also be faced with the problem of biological specialization, as Keener has noted for *Darluca filum* (2).

AS A CONTROL AGENCY

Since cross fertilization by pycniospores (6) appears necessary to the copious development of aecia, then an effective biological control might be found in such an agent as *Tuberculina maxima*, which vigorously attacks

the pycnial stage. The pycnial nectar no doubt harbors many fungi. However, little attention has been paid to these fungi and to their probable effect upon the development of the rust. If a natural agent can be found that destroys large numbers of the pycniospores, and by so doing reduces considerably the aecial production, some measure of control may be had at little cost.

In view of the above, it might prove of value to determine the number and kind of insect carriers of pycniospores. A study of their habits may easily lead to utilizing them in carrying to the pycnial drops small quantities of a chemical that would render the pycniospores functionless. In a similar manner, the insect visitors to pycnial stroma doubtless play an important part in conveying fungus spores other than pycniospores from one canker to another.

In Europe, considerable attention has been given to the possible control of the white-pine blister rust by means of the purple mold. The attempts at control on European forest lands were reported to have greatly reduced and in some cases, inhibited the production of aeciospores (15, 3). The few attempts so far conducted in Idaho have proved ineffective and it is obvious, that no matter what measures of destruction any one biological agency may accomplish under favorable conditions locally or in any specific year; the most effective control of the white-pine blister rust will always be accomplished through the destruction of Ribes plants by mechanical pulling, the various types of chemical spraying, the slashing or bulldozing of brush areas, with subsequent burning, and the control of Ribes population by selective cutting of timbered areas.

SUMMARY

The purple mold, *Tuberculina maxima*, reported as an agency in the control of the white-pine blister rust in Europe, is beginning to be studied in this country.

So little is known of the life history of this fungus that attempts made in 1932 and 1933 to spread it among white pines infected with *Cronartium ribicola* in Idaho met with little success. The purple mold has been grown successfully in cultures but its development is slow and spore production is relatively meager. More work will need to be done before quantity production of the spores can be attained for use in control experiments.

Local, natural establishment of the purple mold and its subsequent spread has been noted but the spread has always been limited to small areas. The possibilities of its use as a biological control agency are remote and can in no way compare with the present mechanical and chemical control methods used in the United States.

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INOCULATIONS OF STAGONOSPORA CURTISII ON THE AMARYLLIDACEAE IN CALIFORNIA[1]

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(Accepted for publication June 13, 1934)

INTRODUCTION

The family Amaryllidaceae contains a number of desirable ornamentals, such as the different forms of Narcissus, Amaryllis, Hippeastrum, Crinum, Leucojum, and other species not so well known. This paper describes some inoculations with *Stagonospora curtisii* (Berk.) Sacc. on these and other species of the Amaryllidaceae grown in California.

THE DISEASE

In a recent paper, Creager (3) has studied the leaf scorch of Narcissus and reports it as having economic importance in the eastern United States. McWhorter and Weiss (9) list the disease in Oregon, while Dougherty (4) and Smith (14) have studied a similar disease in California on Hippeastrum. Other investigators (5, 6, 11, 15) have reported it as present in Europe. Seaver has described (13) *Phyllosticta hymenocallidis* Seaver as attacking *Amaryllis purpurea* Ait. in Louisiana and *Hymenocallis* sp. in Bermuda and in the Greater Bahamas. Petrak (11) describes carmine spots on the leaves of Amaryllis and attributes their cause to *Stagonospora crini* Bubák and Kabát. He records successful inoculation on *Zephyranthes rosea* with *S. crini*. Nicolas and Aggery (10) describe blood red spots on living leaves of *Crinum longifolium* and *C. asiaticum*, and believe them to be caused by *S. crini*.

From the fact that the fungus has been found fruiting on the outer terminal end of the bulb scales, it seems apparent that the leaves may become infected near their tips at the time they are pushing out from the bulb. The disease in California is characterized by red spots on the leaves, flower stems, and sometimes on the petals of *Hippeastrum* sp. The leaf spot is bright red to purple red (blackish red purple).[2] Although small at first, the spots often increase to 50 mm. or more. Pycnidia are apparently developed sparingly in the diseased spots under our climatic conditions, but become more abundant when the diseased leaves are kept in a moist chamber.

On Narcissus no red spots are formed, but the infected areas are brownish and occur on leaves and flower stems. The tissue above and below the

[1] Paper No. 303, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

[2] Ridgway, Robert. Color standards and nomenclature. Washington, D. C., 1912.

infected areas becomes yellow, and the leaf soon dies. The ends of the leaves are the parts most often infected.

In California the disease on Hippeastrum is of only minor importance during seasons of normal rainfall. Some spotting occurs on leaves scattered throughout the field, but no great amount of killing of foliage has been observed. In exceptionally rainy seasons the injury is increased and nearly every plant may have one or more spots. Dougherty,[3] after a careful study of the occurrence of the disease, reported 90 per cent of the plants as being infected with one to several spots. The fungus rarely attacks *Amaryllis belladonna*. The disease is of more importance on Narcissus and may attack and kill plants long before the end of their normal growing season.

SOURCE OF MATERIAL

The cultures used in this study were isolated from stem lesions, leaf spots, and bulb scales of *Hippeastrum* sp., from reddish brown areas on leaves of *Crinum powelli*, from the pycnospores of *Stagonospora curtisii*, and from diseased tissue of Narcissus.

Through the courtesy of H. S. Cunningham, formerly of the Department of Agriculture, Bermuda, the author received some fresh material of *Hymenocallis littoralis* (Jacq.) Salisb., affected with *Phyllosticta hymenocallidis* (13). Some of this diseased tissue was used in inoculating, through wounds, healthy leaves of *Hippeastrum* sp. In the 2 trials made, typical red spots were formed at the point of inoculation, and from this infected tissue a Phyllosticta-like fungus was isolated that was pathogenic on forms of Hippeastrum, Narcissus, and other species of the Amaryllidaceae. (See Table 1, inoculations by cultures from *Hymenocallis* sp.). Attempts to isolate *Phyllosticta hymenocallidis* directly from the diseased leaf tissue of *Hymenocallis littoralis* were unsuccessful, probably because the leaves were too old. The indirect method of using the diseased tissue of *H. littoralis* as an inoculum admittedly leaves some doubt as to whether it is the above fungus or something else in this tissue that causes the infection on Hippeastrum. The remaining plants of Hippeastrum, however, remained free from any natural infection.

THE CAUSAL ORGANISM, ITS SYNONOMY AND DESICCATION

Stagnospora curtisii is the cause of the leaf scorch of Narcissus and the red leaf spot of *Hippeastrum* sp. The fungus is readily isolated by the usual methods and grows well on cornmeal agar, glucose potato agar, and sterilized leaves of Narcissus, Hippeastrum, and Iris. It fruits scantily on the agar media, but pycnidia are abundantly formed on sterilized leaves.

[3] Dougherty, P. I. The red leaf-spot of Hippeastrum. (An unpublished thesis submitted for the degree of Bachelor of Science, Department of Agriculture, University of California, 1914.)

TABLE 1.—*Inoculation on species of the Amaryllidaceae through wounds by spores and mycelium of Stagonospora curtisii*

Hosts inoculated[a]	Sources of cultures used in inoculations[b]				Radius of lesions
	Hippeastrum	*Narcissus*	*Hymenocallis*	*Crinum*	
	Number of lesions				
					mm.
Amaryllis belladonna	3	3	3	2	2–5
**Chlidanthus fragrans*	5	10	6	5	5–10
**Crinum powelli*	3	3	4	7	10–20
**Galanthus* sp.	3	2	4	5	5–10
Hippeastrum vittatum	35	40	5	3	5–50
**Hymenocallis calathina*	2	2	4	2	10–20
**Leucojum vernum*	5	4	11	5	5–15
**Lycoris squamigera*	3	2	3	5	5–20
Narcissus sp.	7	14	2	2	5–40
**Pancratium maritimum*	4	3	6	3	5–10
**Sternbergia lutea*	2	3	3	2	5–20
**Zephyranthus candida*	2	5	3	3	3–10

[a] An asterisk indicates that the species has not before been reported as a host of *Stagonospora curtisii.*

[b] Cultures were isolated from *Hippeastrum* sp., which had been inoculated with tissue of *Hymenocallis littoralis* infected with *Phyllosticta hymenocallidis* Seaver. Cultures were secured from the diseased tissue of species of Hippeastrum, Narcissus, and Crinum.

The spores in artificial cultures are especially variable, as has been noted by Creager (3) and by Smith (14). They are usually small, continuous, or occasionally uniseptate, and could be classified as belonging to the genera Phyllostieta or Phoma. At other times, however, the spores in cultures growing on sterilized leaves of Narcissus or Hippeastrum may be large, 3–5 septate, and typical of *Stagonospora curtisii;* but, even in these cultures, the continuous and uniseptate spores are to be found. The vegetative growth of the fungus may be scant when pycnidia are in great abundance. This variability of spores has been found in all the cultures studied, even though they originated from large, septate spores.

The spores of *Stagonospora curtisii* showed some resistance to desiccation, as shown by the following tests. Dried herbarium material of *S. curtisii* on narcissus leaves was kept in the laboratory at room temperature in paper packets for one year. Dilution cultures made of the spores showed nearly 100 per cent germination. An attempt to germinate the spores from this same herbarium material after 2 years gave negative results. The protection from rapid desiccation of spores is probably largely due to the surrounding pycnidial wall.

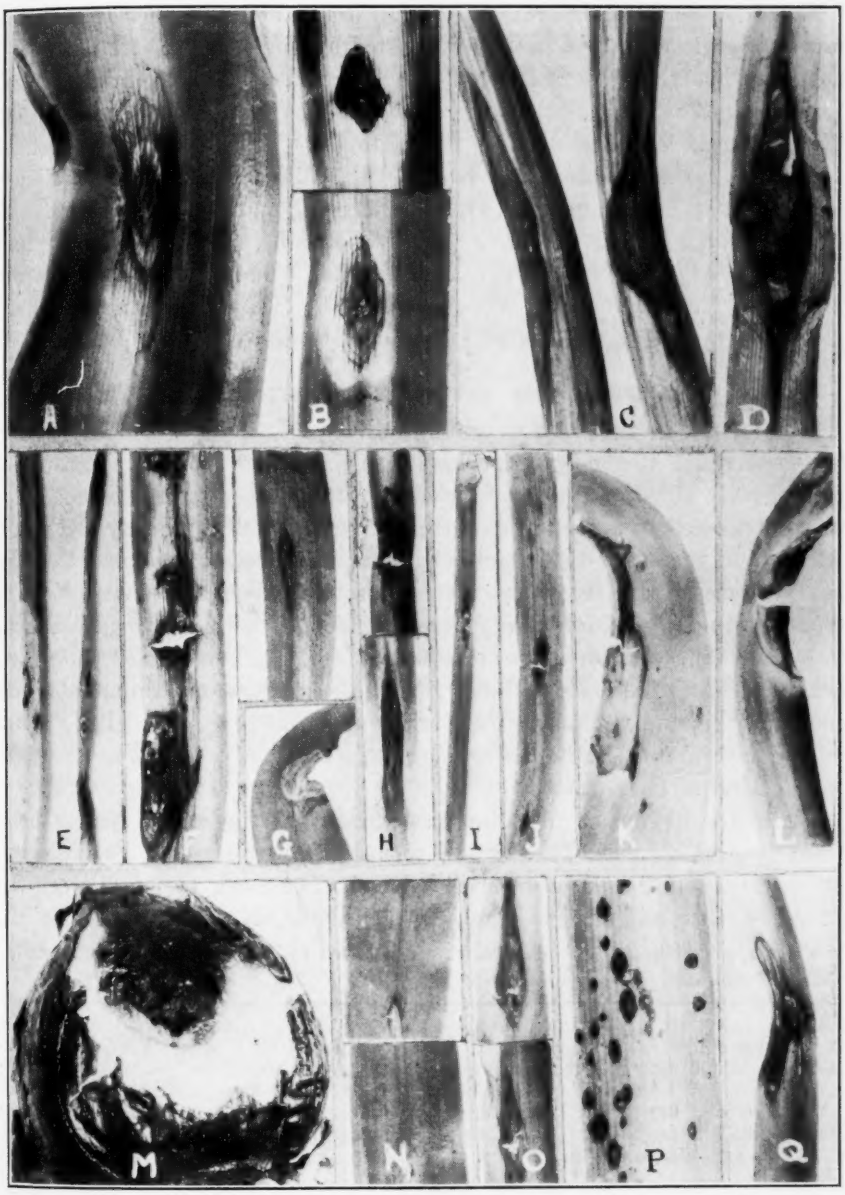

FIG. 1. A. Artificial inoculation on leaf of *Crinum powelli* with culture from *Hippeastrum* sp., infected with diseased tissue from *Hymenocallis littoralis*. Numerous pycnidia on spots. B. Artificial inoculation on leaves of *Hymenocallis calathina* (*Ismene calathina*) through wounds using culture isolated from *Crinum powelli*. Photographed after 5 days. C. Inoculations through wounds on flower stems of *Hippeastrum* sp., using culture isolated from flower stalk of *Hippeastrum* sp. Photographed after 20 days. D. Inoculation on *Pancratium maritimum* by culture from *Hippeastrum* sp., after 15

Narcissus sp., and hybrids of *Hippeastrum vittatum* have long been known to be attacked by a fungus attributed by different workers to Phyllosticta, to Phoma, and to Stagonospora. The names of the following fungi also listed by Creager (3) are believed to be synonyms of *Stagonospora curtisii: Phyllosticta oudemansii* by Saccardo (12), *P. narcissi* by Aderhold (1), *Stagonospora narcissi* by Hollós (6), all occurring on Narcissus; *Phyllosticta* sp. by Dougherty (4), and by Horne and Essig (7), *P. gemmipara* by Zondag (15), *Phoma amaryllis* by Kotthoff and Friedrichs (8), and *Stagonospora* sp. by Petrak (11) on Amaryllis. The writer believes, from his studies, that *Phyllosticta hymenocallidis* by Seaver (13), and *Stagnospora crini* by Bubák and Kabát (2), should be added to this list.

ARTIFICIAL INOCULATIONS ON AMARYLLIDACEAE

Most of the inoculations were through wounds, usually a needle puncture underneath the inoculum, which was prevented from drying out by covering with adhesive tape or Johnson's nurseryman's tape. Inoculations also were made by placing the inoculum in contact with the uninjured tissue and keeping the plant in a moist chamber. The following species of the Amaryllidaceae have been inoculated through wounds:[4] *Amaryllis belladonna* Linn., **Chlidanthus fragrans* Herb., **Crinum powelli* Hort., **Galanthus* sp., *Hippeastrum vittatum* Herb., **Hymenocallis calathina* Nichols (*Ismene calathina* Herb.), **Leucojum vernum* Linn., **Lycoris squamigera* maxim., *Narcissus tazetta* Linn. (Chinese Sacred Lily), **Pancratium maritimum* Linn., **Sternbergia lutea* Ker-Gawl., **Zephyranthes candida* Herb. (Fig. 1.)

While the degree of infection has varied, all the above species have shown positive lesions. The results from atomizing spores on *Narcissus* sp. and on *Hippeastrum* sp. were also positive. The more susceptible are the species of Crinum, Hippeastrum, Hymenocallis, Narcissus, Pancratium,

[4] The species marked with an asterisk are believed to have been hitherto unreported as hosts of *Stagonospora curtisii*.

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days. E. Leaves of *Zephyranthes candida* inoculated with culture from *Crinum powelli*, left, and from *Hippeastrum* sp., right. Photographed after 4 days. F. *Lycoris squamigera* inoculated with culture from *Hippeastrum* sp., after 15 days. G. *Galanthus* sp.: Above, inoculated with culture from *Narcissus* sp., after 20 days; below, from *Hippeastrum* sp., after 15 days. H. Inoculation of *Sternbergia lutea:* Below, with culture from *Crinum powelli*, after 20 days; above, from *Narcissus* sp., after 25 days. I. Inoculation on *Chlidanthus fragrans*, after 20 days, with culture from *Narcissus* sp. J. Inoculation on *Pancratium maritimum* with culture from *Narcissus* sp., after 20 days. K. *Hippeastrum* sp. inoculated with culture from *Narcissus* sp. Photographed after 30 days. L. *Lycoris squamigera* inoculated with culture from *Hymenocallis* after 15 days. M. Narcissus bulb inoculated with culture from *Narcissus* sp. Photographed 9 months later. N. *Amaryllis belladonna* inoculated: Above, with culture from *Crinum*; below, from *Narcissus* sp. Lesions small. *A. belladonna* seems to show some resistance. O. *Narcissus* sp. inoculated: Above, with culture from narcissus; below, from Hippeastrum. P. Natural infection on *Hippeastrum* sp. with *Stagonospora curtisii*. Q. Inoculation on *Leucojum vernum* by culture from Hymenocallis after 15 days.

Chlidanthus, Sternbergia, Lycoris, and Leucojum. *Zephyranthes candida*, when infected, developed a small lesion, and the tissue of leaf above and below the lesion became a bright yellow. *Amaryllis belladonna* was very slightly infected (1–2 mm.) when inoculated through a wound. Dougherty also failed to get significant results in his inoculations on *Amaryllis belladonna*, but he was successful in his inoculations of the Amazon lily, *Eucharis grandiflora* Planch, and *Hippeastrum* sp. In the following species of the Liliaceae no lesions developed from inoculations: *Agapanthus umbellatus* L. Her., *Allium neapolitanum* Cyr., *Convallaria majalis* Linn. (lily-of-the-valley), *Hemerocallis aurantiaca* Baker, and *Ixia* sp. Inoculations on *Iris germanica* Linn. were also negative.

SUMMARY

A disease of Narcissus and Hippeastrum caused by *Stagonospora curtisii* is described as occurring in California. It was found to be pathogenic on the following species of the family Amaryllidaceae: *Amaryllis belladonna, Chlidanthus fragrans, Crinum powelli, Galanthus* sp., *Hippeastrum* sp., *Hymenocallis calathina, Leucojum vernum, Lycoris squamigera, Narcissus* sp., *Pancratium maritimum, Sternbergia lutea, Zephyranthes candida.* The spores are variable in size, shape, and septation. In the same pycndium may be found large, 1–5 septate spores and smaller, continuous ones, although in cultures nearly all of the spores may be continuous. Apparently the pycnidium can protect the spores for some time from desiccation. In some tests of dry herbarium material, spores were viable after one year.

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MACROSPORIUM AND COLLETOTRICHUM ROTS OF TURNIP ROOTS

CHARLES CHUPP

(Accepted for publication July 10, 1934)

Macrosporium herculeum E. and M.

In the early spring of 1932 specimens of diseased turnips were received from Columbus, Ohio, with the statement that the trouble affecting them was important in that part of the State. It had been observed first on the foliage of the crop during the warm, moist weather of the previous August. Later, a majority of the leaves dropped except a small central green cluster. Simultaneously with leaf spotting the roots became affected, so that many plants died and rotted completely. The remaining roots grew no larger and were unmarketable at the end of the season. Later planted turnips became affected also, but to a much less degree. When the roots of this late crop were placed in pits, the high winter temperature apparently favored the growth of the fungus, for most of the crop thus stored was destroyed.

The specimens sent to the laboratory showed circular lesions, usually consisting of two or more zones. The central part of each spot was com-

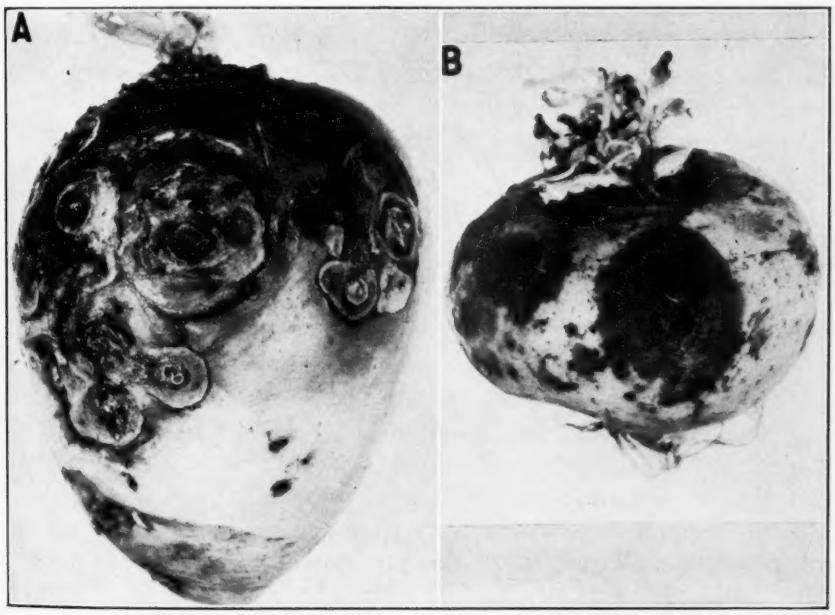

FIG. 1. A. Lesions caused by *Macrosporium herculeum* on field turnip. B. Turnip root 20 days after inoculation with *M. herculeum*.

posed of rather dark brown tissue or was whitened by aerial growth of mycelium. This area was bounded by a narrow dark band (Fig. 1, A). Lesions varied from no band to several, and from one uniform brown shade to several shades and sometimes even almost black. The spots never seemed to be depressed nor the affected tissues watery.

In isolations, the first plantings in nearly every case gave pure cultures of Macrosporium. These cultures were abundantly supplied with conidia, the shape and size of which compared closely enough with the illustrations and measurements given by Weimer (5) and earlier workers (Stewart 3, Ellis and Martin 1) to consider the organism identical with *Macrosporium herculeum* E. and M. (or Alternaria for those who divide the genus). Weimer's drawings show slightly thicker beaks, as compared with the width of the spore body, than do those from the Ohio material (Fig. 2, D). His measurements are $16–28 \times 125–225\ \mu$; spores from naturally infected turnips and from cultures measured $11.4–24.7 \times 79.8–342.0\ \mu$, with an average of $17.5 \times 143.6\ \mu$ for a hundred spores.

Since the fungus had not been reported as causing serious root trouble, inoculations were made on leaves and roots with spore suspensions, and into roots with mycelium. Leaves uninjured by pricking showed infection before injured ones. Visible infection was present on the leaves eight days after inoculation (Fig. 2, A). Under moist greenhouse conditions the parts between the spots soon withered, and the whole leaf died. The Macrosporium fruited slightly on these spots and the conidia were identical with those from the original specimen. Reisolations from these spots also were made with ease.

A spore suspension dropped on turnip roots in moist chambers or bits of mycelium from agar laid on the uninjured root surface produced infection very slowly under laboratory conditions. But when the epidermis was injured with a scalpel and the inoculum placed against the exposed tissue, one hundred per cent infection was obtained, while checks remained healthy. Figure 1, B shows a turnip twenty days after inoculation. Under moist-chamber conditions the lesion is uniformly dark brown, slightly sunken, and wetter in appearance than lesions caused by natural infection. In such an infected root, cut open, the rot extends almost to its center, is uniformly brown throughout the invaded tissue, and at its edges gradually blends with the white of the normal area. The rot is darker colored than that caused by *Erwinia carotovora* and is not so soft in texture.

Turnip seeds, as represented by varieties and strains bought in the open market, almost uniformly show the presence of *Macrosporium brassicae* Berk. (Alternaria) when plated on agar. An occasional lot of seed may show *Macrosporium herculeum*, but apparently never in large amounts and then mixed with the former species. The two species can readily be told

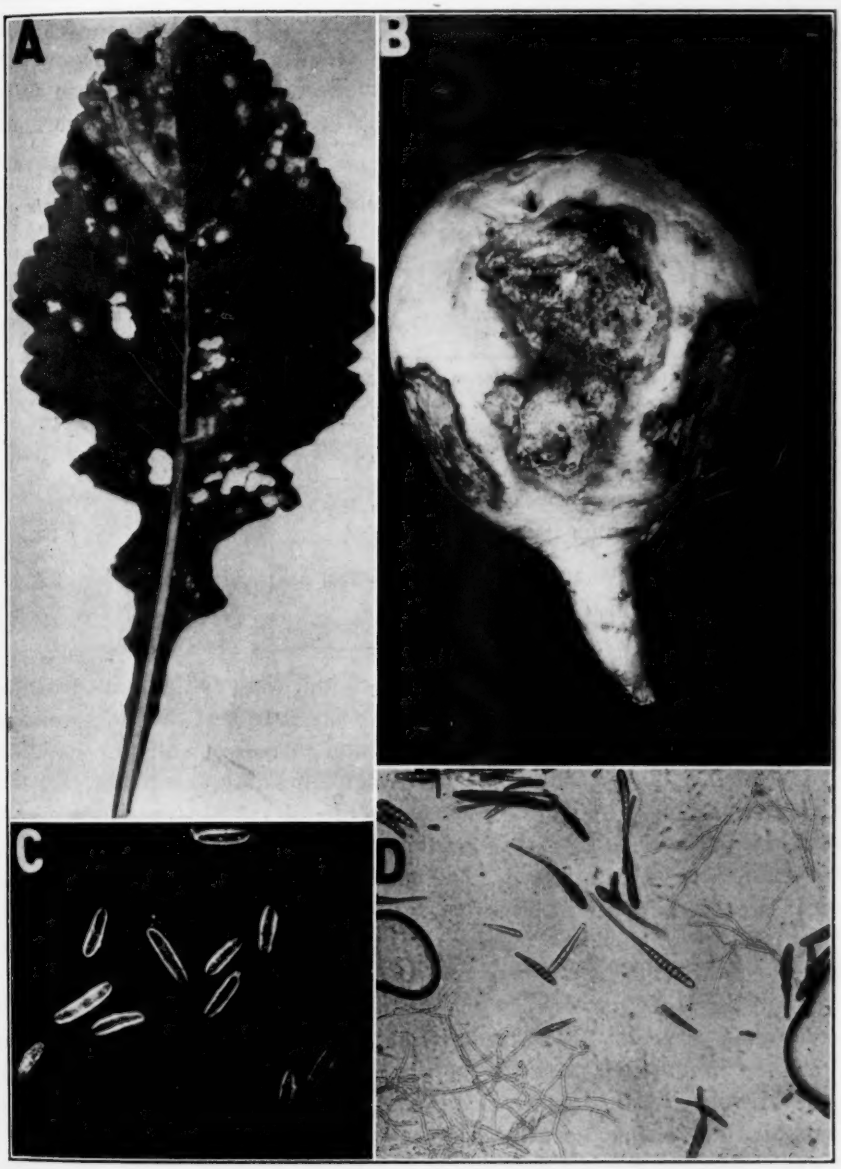

FIG. 2. A. Leaf 8 days after inoculation with Macrosporium from diseased turnip roots. B. Turnip root naturally infected with *Colletotrichum higginsianum*. C. Spores of *C. higginsianum* from turnip root. ×500. D. Spores of *M. herculeum*. ×60.

apart, as shown by Weimer's drawings (b, c). Preliminary tests showed the two species to be approximately equal in their resistance to heat and fungicides. Therefore, in the following seed-treatment experiments, the *Macrosporium* infected seed counts were made without attempting to determine the relative species numbers. The seeds used in the experiment showed the presence of both fungi.

TABLE 1.—*Effects of various seed treatments in reducing the number of seeds producing Macrosporium colonies on agar plates*

No. of seeds plated	Treatment	Length of treatment	No. showing Macrosporium growth
108	Calcium hypochlorite, decanted solution	2 hrs.	62
109	" " " "	2.5 hrs.	15
55	" " " "	1.5 hrs.	8
157	Soaked in water 20 hrs., treated in $HgCl_2$–1–1000, then rinsed in 70% alcohol	3 min.	46
111	$HgCl_2$–1–1000 and rinsed in 70% alcohol	25 min.	28
1706	Hot water 50° C.	10 min.	1

Check plates, without treatment of any kind, were contaminated with so many organisms that it was impossible to make counts of the Macrosporium infected seeds. Apparently hypochlorite and corrosive sublimate could not be depended upon for controlling the fungus, possibly due to the fact that the mycelium grows under the seed coat, although not observed to penetrate farther into the seed, for, when portions of the naked seed were macerated and stained, no mycelium could be found. Furthermore, if seed is soaked until the seed coats can be slipped off, the naked seed treated for 3 minutes in corrosive sublimate and rinsed in 70 per cent alcohol before plating on agar, no growth of Macrosporium appears on the media.

The Macrosporium is very susceptible to injury from hot water. In preliminary experiments three-minute treatments at 50° C. apparently killed the fungus in a majority of the seeds. This short treatment, however, does not kill the surface bacteria, so that Petri-dish platings were too contaminated to make accurate counts.

No opportunity was available for studying the effect of hot water seed treatment on field control of the disease.

Colletotrichum higginsianum Sacc.

In March, 1932, specimens of diseased turnips were received from Rochester, N. Y. These cold-storage turnips had been recently washed and

packed for market. They were of the elongated white type and on each were two to five lesions.

The rot begins on the roots as a small, dry sunken point, and as it enlarges, the affected tissue becomes dirty grayish or light tan in color. The larger the spot becomes, the more sunken the tissue, the margin being abruptly depressed and fairly regular (Fig. 2, B). The older spots may have a slightly wrinkled margin, particularly if bacterial soft rot follows the Colletotrichum. The bacteria apparently gain entrance at the margin of the lesion and soon encircle it with a light- to tobacco-brown, wet-appearing rot, which finally may include the whole root. Penicillium may grow over the dry lesion, but it is very noticeable that it does not extend into the tissue thoroughly invaded by the bacteria.

If an inoculated root is placed in a moist chamber, the infected part is sparingly covered with aerial mycelium. No fruiting bodies are visible to the unaided eye, but in mounts of mycelium scraped from the root, a large number of Colletotrichum conidia are found (Fig. 2, C).

These conidia compare very closely in size to those described by Higgins and to those of the fungus named *Colletotrichum higginsianum* by Saccardo, the measurements being 2.5–4 × 15–20 μ with an average of 3.2 × 18.1 μ for the conidia of the Colletotrichum on turnip, while Saccardo records 3–3.3 × 15–17 μ, and Higgins 4–5.5 × 13.5–19.5 μ for those of the fungus discovered in Georgia. They are perfectly hyaline, cylindrical, with rounded ends, and occasionally with a marking that has the appearance of a cross wall.

A suspension of these spores sprayed on a turnip leaf, or placed on the exposed tissue of the turnip root, infects readily, producing lesions on the foliage similar to those on the type material of *C. higginsianum*, which Higgins sent for comparison, and on the roots lesions similar to those found on naturally infected turnips.

Higgins, in his publication regarding the fungus (2), makes the statement that " . . . during two previous seasons the disease had been very destructive to young turnip (leaves), but observations on specimens sent from this field indicate that the greater part of damage was probably due to a bacterial soft rot of the roots." He may possibly have found only the late stages of the Colletotrichum disease after the soft-rot bacterium had overrun the original lesions. On the other hand, this leaf-invading organism possibly affects the roots only under the most favorable condition and therefore is not often present in the field.

The fungus overwinters in partly rotted leaves kept moist among old leaves on the soil and was successfully reisolated from them. No trace of it, however, could be found on or in the seeds, even though numerous plantings were made of disinfected whole seeds and seed coats, as well as coty-

ledons and embryos that had been removed from their outer covering. Unfortunately none of the seed from the stock that grew the diseased crop was available for experimentation, so that the negative results obtained in the attempted isolations may indicate merely that the seed strains available were not infected.

SUMMARY

Macrosporium herculeum and *Colletotrichum higginsianum*, both previously listed as leaf parasites on various cruciferous crops, are shown to cause serious turnip root rots either in the field or in storage.

The Macrosporium is carried on or in the seed and can be killed by seed treatment for 10 minutes in water at 122° F.

The Colletotrichum could not be proved to be carried on or in the seed. The fungus, however, does overwinter in old affected leaves.

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LITTLE LEAF OR ROSETTE OF FRUIT TREES IN CALIFORNIA

ANTONI KOZLOWSKI

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Shoots of peach, apple and plum trees, producing abnormally small leaves and finally destroyed by an exanthema, were investigated in the summer of 1933. From the bark parenchyma of the last season's shoots Monilia was isolated.

Some preliminary experiments on the infection were carried out in August, 1933, and showed positive results.

Twigs with small leaves were marked on the sick trees in the orchards concerned in the autumn of 1933, and were examined in the winter of 1934. The presence of Monilia in the bark of the shoots was established and there is no doubt that Monilia causes a fatal injury of the trees concerned. The infection of the trees is facilitated by the unfavorable environmental conditions, as for instance by the anaerobic soil conditions in the peach and apple orchards in Delhi, Merced County, and in Sebastopol, Sonoma County, respectively, or by sand drought in the plum orchard in Bakersfield, Kern County. In this latter orchard the Japanese plum trees grow in deep sand. The reaction of the soil solution is about pH 8.4 to pH 8.6; there is a deficiency of potassium, nitrates and phosphates at the depth of 3 to 6 feet. The trees are exposed to a severe drought during warm, rainless weather. The shoots collected at the beginning of March of 1934 showed that their winter buds were entirely dry and fell off readily; no parasites could be found in the buds thus injured. The main and the lateral small shoots were decayed at their tips. The scars from the fallen leaves and buds were not healed. On cutting off the epidermis at the base of the scars brown spots could be noticed, which represented decayed fibrovascular bundles connecting the leaf petiole with the stem. Such lesions could be detected at the base of almost all scars of the leaves. In a majority of cases a pure culture of Monilia was isolated.

The soil in the apple orchard investigated is a sandy loam; the reaction of the soil solution was a pH 5.7 to 4.8. There was a deficiency of phosphates at the depth of 2 to 6 feet. Only traces of nitrates were found at the depth of 3 feet. Processes of anaerobic fermentation were found at the depth of 4 feet during winter. A clay subsoil was found at the depth of 5 feet. The apple shoots collected at the end of January, 1934, showed that the meristematic tissues in the terminal buds were entirely destroyed. It

looked as if the meristematic cone were cut off at its base so that the medulla was exposed to the surface. The lesion was covered with hairs; the medulla contained healthy greenish cells with compound crystals of calcium oxalate and showed no infection. The scales of the terminal buds were peeled off so that only a few were left and even these were dstroyed at their tips. The lateral winter buds did not show any injury of their meristematic tissues, which, however, were poorly developed. The hairs of the scales of the terminal buds were covered with hyphae and conidia of Monilia. This fungus lives at first saprophytically on the scales of the buds and gradually penetrates into the bark of the shoots.

Among the hairs of the scales of the terminal buds was frequently found a microscopically small snout mite, apparently a species intermediate between *Notophallus* sp. (Eupopidae), and *Bdella* sp. Its rostrum is thick and shorter than that of Bdella, and shows a long, brown, meridianal line. The mite is light brown, almost transparent, and has a few brown spots at the hind part of its dorsum. Its legs terminate in two simple claws. The palpi are biforked. A few bristles cover the dorsum and the legs. It is possible that this mite is not purely predaceous and may cause the above described destruction of the terminal buds.

The soil in the peach orchard investigated is a slightly loamy sand. The reaction of the soil solution was pH 7.3 to 8.4. Hardpan was found at the depth of 6 feet. Processes of anaerobic fermentation were found at the depth of 5 feet. An accumulation of sodium chloride was found at the depth of 4 feet. The shoots of peach trees collected at the beginning of March, 1934, were in a majority of cases entirely healthy. The winter buds showed no injury. In a few cases, however, brown spots were found in the bark parenchyma just below and on both sides of a bud. From such lesions *Monilia* sp. was isolated and found to be identical with the species isolated last summer from the last season's twigs. The spores of that fungus, although not numerous, were found on the surface of the winter buds.

The infection of the twigs of peach trees seems to progress much more slowly than that of plum trees.

On some shoots an injury of the bark was caused by insects and there were found eggs of the California katydid, *Scudderia furcifera*, attached in rows to the twigs.

The species of Monilia, isolated from the above 3 kinds of trees, seem to be very closely correlated and greatly resemble *Monilia cinerea*.

RESULTS OBTAINED FROM MORE RECENT EXPERIMENTS

The formation of abnormally small leaves in diseased peach, apple, and plum trees studied in California apparently is due to the following 3 factors: anaerobic soil conditions, infection by *Monilia* sp., and climate.

Anaerobic soil conditions found in the orchards of peach and apple trees under investigation, which were grown in a sandy soil with a faulty substratum, hardpan and clay subsoil, respectively, caused serious injury to the roots. The leaves of the trees exposed to such conditions were of normal structure and texture but of subnormal size; they also were chlorotic and mottled when the soil reaction was alkaline. The trees so affected were readily invaded by *Monilia* sp., which caused the leaves of the infected shoots to be small, stiff, and of glassy appearance; they grew in clusters. An infection by *Monilia* sp. was mainly responsible for the little-leaf formation on the plum trees grown in a deep sand, in which the trees were exposed not to anaerobic soil conditions but rather to a severe sand drought.

A formation of a witches'-broom-like growth of the shoots is possibly due not to Monilia but to some other fungus.

A formation of small leaves under the influence of anaerobic soil conditions can be readily reproduced experimentally, above all when the meristematic tissues are exposed to such injury during the rest period of the trees, as may be seen from the following instance: Small apple trees were grown about 18 months in 1-gal. crocks containing a loamy sand that was quite rich in iron oxide and iron hydrate. In one series, the soil was oversaturated with water in the first week of December, and, in another series, during the first week of January. A layer of water about 1 cm. deep covered the soil and became more or less rusty. The water was allowed to evaporate spontaneously. The trees started to develop their leaves in mid-February; the leaves in the first series were small and died in March; those in the second series were small, $\frac{1}{2}$ to 1 cm. long, during March, but they began to increase in size towards the end of April. The soil was kept only slightly moist during the second part of February and the following months. Apparently, the cessation of the anaerobic conditions caused an improvement of the trees in the second series of experiments. The leaves in the controls were 5 to 10 times larger than those in the second series in March and April.

An artificial formation of small leaves during the summer months could be induced by an addition of 300 cc. of a culture medium of the nitrate-reducing Pseudomonas to one gallon of the sand in which the small peach trees had been grown for a few months. The leaves of the trees thus treated fell off in about 10 days; the new ones, formed about 6 weeks later, were very small and the plants died in 2 months.

An experimental reproduction of the formation of small leaves under the influence of an infection by *Monilia* sp. has been attempted with some positive results. This problem, however, demands special investigation with a reproduction of all environmental conditions found in the orchards concerned.

It is very possible that by proper regulation of the anaerobic conditions of the soil one could inhibit the growth of the leaves during the entire summer season.

In conclusion, the author wishes to express his thanks to J. C. Martin and R. O. Overstreet for the soil analysis; to N. D. Hudson, the Assistant County Agent in Bakersfield, Kern Co.; to Enoch Torpen, the Assistant County Agent in Santa Rosa, Sonoma Co., to E. C. Ruehl in Delhi, Merced County, for their cooperation in securing the soil samples and in marking and collecting the shoots of the trees, and to Mr. Pederson for his very kind help in the greenhouse.

PHYTOPATHOLOGICAL NOTES

A Portable Rust-Inoculation Chamber.—In the conduct of field nursery experiments at the Arlington Experiment Farm, Rosslyn, Va., involving a study of host reaction to cereal rusts, it often is desirable to inoculate plants grown from seed obtained from specific head selections, or a limited number of plants from one or more crosses, under highly favorable conditions for rust infection. To accomplish this object, the writers designed a portable compo-board chamber, 8 ft. x 4 ft. x 4 ft., equipped with a cover, 8 ft. x 4 ft. in dimensions.

When the nursery row, or part of a row, selected for inoculation has reached the desired stage of plant development, the chamber is placed over it and pressed down until the lower edge is snugly in contact with the soil at all points. It is then banked with soil to insure retention of humidity within the chamber and prevent a too hasty dissipation of the artificial dew subsequently to be applied by means of an atomized spray of tap water. After the plants are generously sprayed, they are liberally dusted with urediospores mixed with some innocuous white dust, such as powdered talc. The talc powder increases the visibility of the inoculum and thus insures a more uniform distribution of the spores without in the least affecting their germinability or potential capacity to produce infection.

To obtain the maximum infection, the inoculum should be applied in late afternoon, provided the day be warm and sunny. If the sky be overcast,

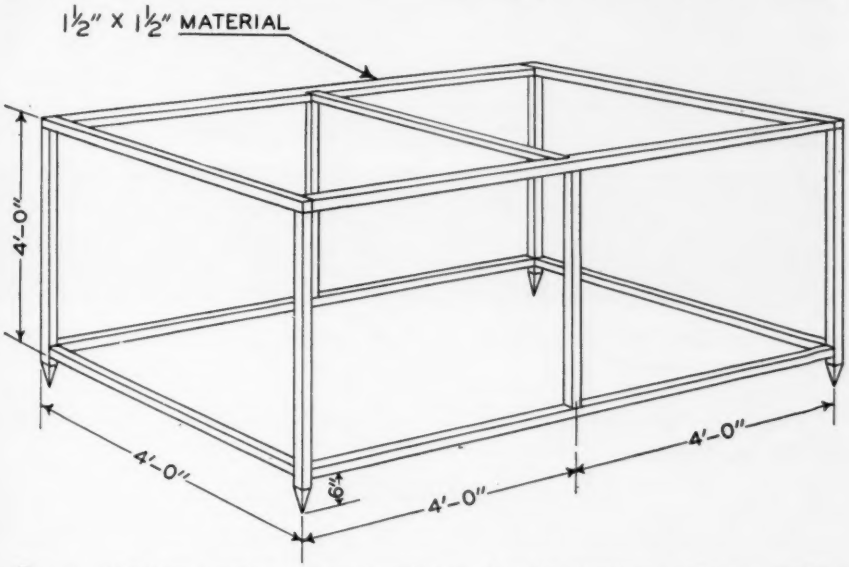

FIG. 1. Working drawing of rust-inoculation chamber showing details of construction of the frame.

the spore shower may be administered earlier in the day. The cover of the chamber is then put in place and the spores are allowed to incubate 24 hours or longer, when the cover is removed and the chamber is reset for a repetition of the operation. Its lightness renders it easily portable by two men.

By means of the inoculation chamber and inoculation technique here described, the writers have been able to subject to individual test in the field a large number of selections and varieties of oats (Fig. 2), under meteorologic conditions quite inimical to the natural development and spread of infection. Moreover, it has enabled them to subject these selections and varieties to infection by known physiologic forms of crown rust (*Puccinia coronata*) and stem rust (*P. graminis avenae*) of oats.

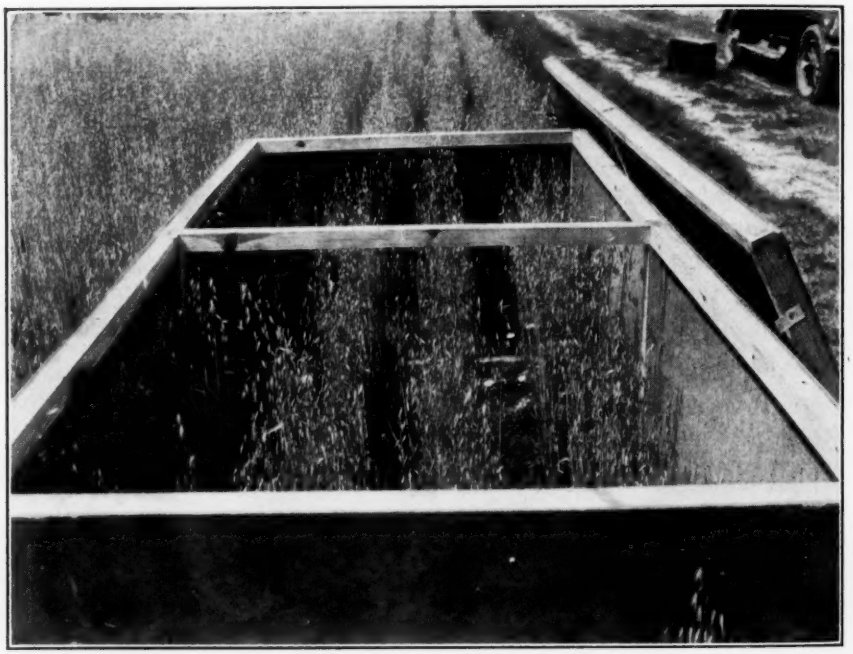

FIG. 2. Inoculation chamber, with cover removed, showing rust-inoculated portions of four nursery rows of oats.

Following is a description of the inoculation chamber and specifications for its construction.

Specifications

This inoculation chamber consists of a rectangular compo-board box covered by a lid of the same material. The frame for supporting the compo-board is made of dressed pine, approximately 1½ in. square, put to-

gether with No. 12 casing nails. The compo-board is attached to the frame with shingle nails, its smooth surface outside (Fig. 2).

The box is 8 ft. long, 4 ft. wide, and 4 ft. high to the cover. The cover is 8 ft. long by 4 ft. wide.

Frames for the ends are made first. The legs or corner posts are $4\frac{1}{2}$ ft. long, sharpened at the lower end to facilitate pressing them into the soil and thus insuring stability of the chamber in the event of a windstorm. The upper or top crosspiece for each end is nailed on top of the legs, and the lower crosspiece of each end between them so that the distance between these 2 crosspieces is approximately $3\frac{3}{4}$ ft. and the over-all distance is 4 ft. The compo-board is then nailed to each end frame. After the ends are completed, they are connected by 4 longitudinal supports, 1 each at the top and bottom on each side. These supports are $7\frac{2}{3}$ ft. long and are toe-nailed into the upright corner posts, their outer edges being kept flush with those of the top and bottom crosswise end braces of each end frame.

To further strengthen the chamber an upright brace is nailed between the longitudinal supports midway on either side and a third brace is nailed crosswise of the box midway between the 2 top longitudinal supports. The compo-board sides are then nailed in place so as to cover the edges of the pieces of compo-board covering the ends.

The cover is made of one piece of compo-board, 8 x 4 ft., attached to an oblong pine frame, 4 x 8 ft., outside dimensions. This frame is made of $1\frac{1}{2}$ in. dressed pine. To add strength to the cover, a brace is placed across it midway between the ends. To secure the cover against disturbance by wind, a hook is placed outside the box on either side near the bottom of the upright middle brace and a clothesline rope is then drawn over the box and tied to these hooks.

If the soil be too hard, it sometimes is necessary to loosen it with a spade in order that the corner posts may be pushed into the ground. A few taps with a maul on the top of each corner post will settle the chamber to a position of complete contact with the soil. Any openings under the bottom of the chamber can be remedied with a spade so as to stop any vents.—H. B. HUMPHREY and F. A. COFFMAN, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

Losses From Corn Ear Rots in the United States.—In an earlier paper[1] attention was called to certain of the estimates of losses from plant diseases in the United States compiled by the Plant Disease Survey that seemed especially worthy of consideration. In two of these cases the probable accu-

[1] Stevens, Neil E. Some significant estimates of losses from plant diseases in the United States. Phytopath. 23: 975–984. 1933.

racy of the Survey estimates was attested by their close correspondence with some other method of measuring disease losses.

A third instance of this appears in the estimates recently compiled by the Survey of losses from corn ear rots from 1922 to 1933 shown in figure 1. Information as to losses from corn ear rots, over aproximately the same period but derived from a wholly independent and quite different source, is also given in figure 1.[2] This information, compiled by the Grain Division of the Bureau of Agricultural Economics, gives the annual variation in "damaged kernels" expressed as percentage of cars showing more than 6 per cent total damage in the check inspections made by over 40 offices of Federal Grain Supervision. All observers are agreed that ear and

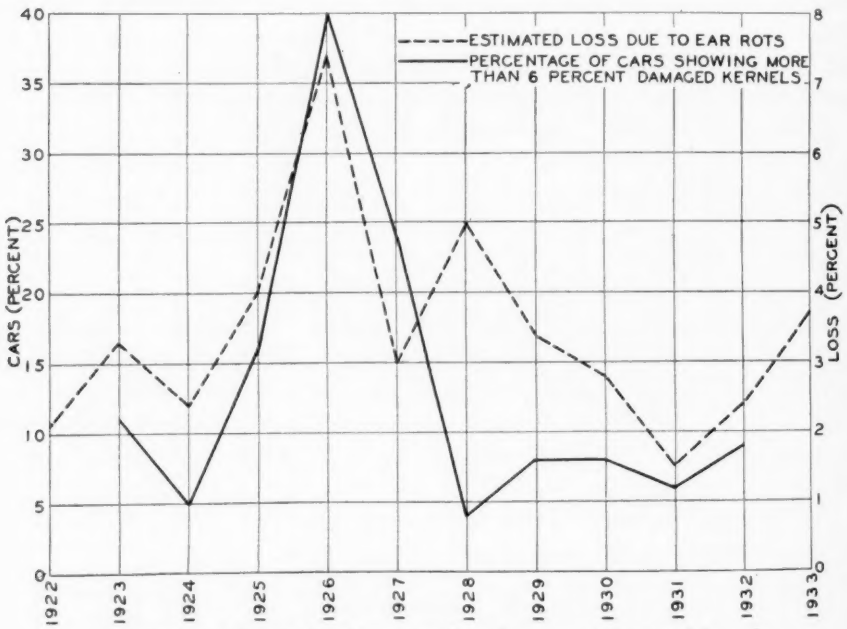

FIG. 1. Percentage of cars of corn, showing more than 6 per cent damaged kernels, as indicated by reports of Federal grain inspectors of the Bureau of Agricultural Economics, for the years 1923 to 1932, inclusive. (After graph published by Grain Standards Educational Committee.[2])

Estimated losses in corn due to ear rots for the United States as a whole, and compiled from reports from collaborators of the Plant Disease Survey.

[2] Corn: Annual variation in the principal factors grading receipts lower than No. 3, Nov. 1, 1923, to Oct. 31, 1932. Graph on page 10 of Grain Inspector's Letter. Issued from General Field Headquarters Grain Division, Bureau of Agricultural Economics, Department of Agriculture, by the Grain Standards Educational Committee. Dec. 19, 1932.

kernel rots are very largely responsible for the damaged corn included in the grade factor "total damage."[3]

The correspondence of the major features of these two curves, one based wholly on field observations and the other on inspections at terminal markets, is obvious. Both show a low point in 1924, followed in 1926 by the largest losses of the period, and relatively much smaller losses in the last 4 years, with a low point in 1931. The chief disparity between the curves appears in 1928.

The close agreement between these two sets of figures, derived from such totally unrelated sources, strengthens the probability that both give fairly accurate pictures of the major fluctuations in losses from corn ear rots in the United States.—NEIL E. STEVENS and JESSIE I. WOOD, Bureau of Plant Industry, Washington, D. C.

Joined Spores of Ascochyta viciae.[1]—In an examination of pycnidia of *Ascochyta viciae* Lib., produced on autoclaved wheat straw, it was observed repeatedly that the spores exuded from them in a mass and did not separate freely in water. Vigorous pressure applied to the cover slip so as to flatten the spore masses failed to effect their separation. They were found to be held together by short tubes, about 2 μ long, and of the same diameter as the germ tubes. At first, it appeared as though a spore had developed a short germ tube, which in turn had given rise to a second normal pycnospore. If such a process were repeated successively, it is possible that a chain or group of 15 or 20 joined spores could be formed. However, the spore masses were observed in pycnidia within 3 days after their appearance and within 13 days after subplantation; so it would seem that a theory of successive spore germination could not account for this phenomenon. It is considered more likely that the spores were formed in this condition from the basal cells or conidiophores of the pycnidium.

The tubes showed variation as to their points of attachment and the spores were connected according to no definite pattern in the following manners: (1) from region of septum to a similar location of another spore, (2) from distal end to distal end of a second spore, and (3) from region of septum of one spore to distal end of another spore. For this reason a compact mass rather than a long chain of spores was formed.

Cultures up to the age of 10 weeks were examined and still showed some of these spore unions, although in lesser amounts than did the younger cultures. Growths on oat and rye straw, arising from the same strain of

[3] Holbert, J. R. Plant breeders make progress in developing disease-resistant corn. U. S. Dept. Agr. Yearbook 1934: 285–287. 1934.

[1] Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 48.

Ascochyta viciae (N. Y. No. 107) and developed under an identical environment, contained only normal free pycnospores. Moreover subplants of this strain cultured on wheat straws from other fields developed only an occasional spore union.

In the expectation that this phenomenon would be observed again the original wheat-straw medium was reautoclaved and subplantation was effected with strain N. Y. No. 93. Fungal growths developed promptly and pycnidia formed profusely. A few of these contained the described joined spores but by no means as plentifully as in the first mentioned. Seven strains of *Ascochyta viciae* recently isolated from imported vetch seeds were cultured on autoclaved wheat straw from several fields. A careful examination of 500 pycnidia did not reveal any spore unions. The same strains were cultured also on autoclaved stems of white-blossom sweet clover, winter vetch, white kidney bean, yellow trefoil, and autoclaved oat and rye straw. No joined spores were observed in the resultant pycnidia.—WILLARD CROSIER, New York State Agricultural Experiment Station, Geneva, N. Y.

Nuclear Phenomena in Helminthosporium gramineum.—Recently, Christensen and Graham[1] demonstrated that *Helminthosporium gramineum* Rab. comprises numerous cultural and parasitic races. They found also that new races arose frequently in culture either as sectors or "patch" variants, as well as in or on the living host. As an aid to the elucidation of the nature of variation in this species, the writer has made cytologic studies of various growth stages of the pathogen.

Of the various killing and fixing solutions used, Flemming's weaker was one of the most satisfactory. Iron-alum haematoxylin was used for staining.

The mycelium produced in artificial culture was stained and examined. In addition, preparations were made from seed coats and endosperms of seed infected as a result of artificial inoculation, from diseased seedlings before the emergence of the young leaves from the coleoptile, and from mature diseased leaves. The cells of the hyphae were almost universally multinucleate (Fig. 1, B). Macroconidia and microconidia, which were occasionally produced in chains from the macroconidia, also were multinucleate, each cell usually containing 4 to 7 nuclei, although the range in number was from 1 to 13. The germ tubes are, of course, also distinctly multinucleate.

The origin of the several nuclei in a cell is an important consideration in surmises on their possible effect in variation in the fungus. Observations indicate that they probably were not derived from a single original nucleus.

[1] Christensen, J. J., and T. W. Graham. Physiologic specialization and variation in *Helminthosporium gramineum* Rab. Minn. Agr. Exp. Sta. Tech. Bul. 95, 1934.

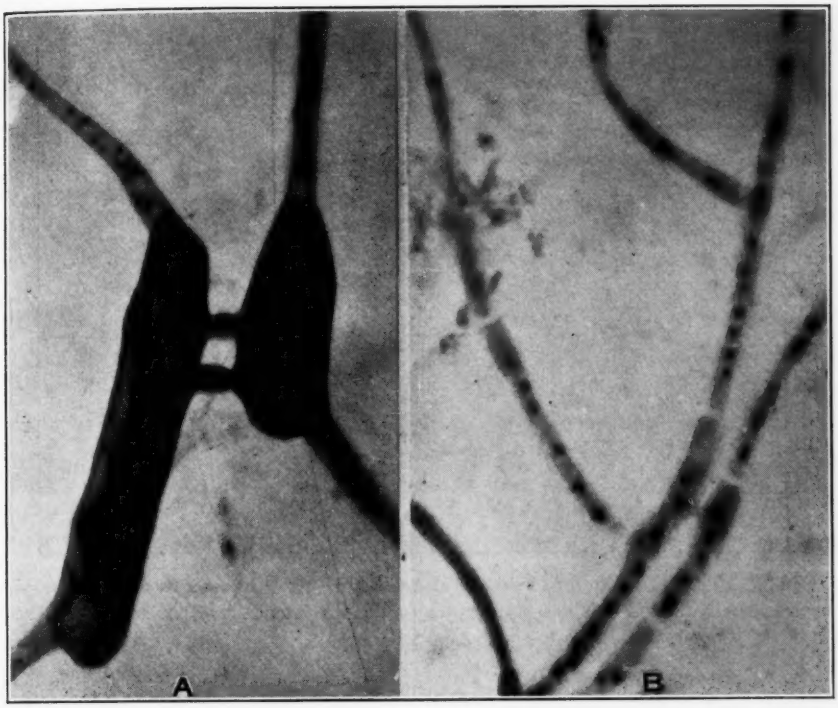

FIG. 1. A. Photomicrograph of two conidia of *Helminthosporium gramineum*. Two fusion tubes connect two different cells of each conidium. Nuclei are passing through the fusion tubes. The nuclei have been retouched slightly to bring them out more clearly. B. Photomicrograph of hyphae showing multinucleate cells. Fusion between two hyphae on the right.

Figure 2 shows quite clearly that the very young spore already contains several nuclei; hence it is quite possible that each may carry different factors for cultural characters and pathogenicity. The organism may then be truly heterocaryotic.

Not only are the cells of the conidia and mycelium multinucleate, but there may be interchange of nuclei between them. Conidia, germ tubes, and hyphal cells often fuse, as shown in figure 1, A and B. Evidently, nuclei pass through the fusion tubes from one cell to another, thus still further increasing the complexity of the nuclear complement of cells.

The evidence indicates strongly that heterocaryosis may account, at least in part, for variation in *Helminthosporium gramineum* and the production of new races. Even if all of the nuclei in the cells contained the same factors, which they probably do not, the differences in number easily could account for some differences in characters of different cells. The cells are multinucleate at the beginning, and nuclear interchange is common. It

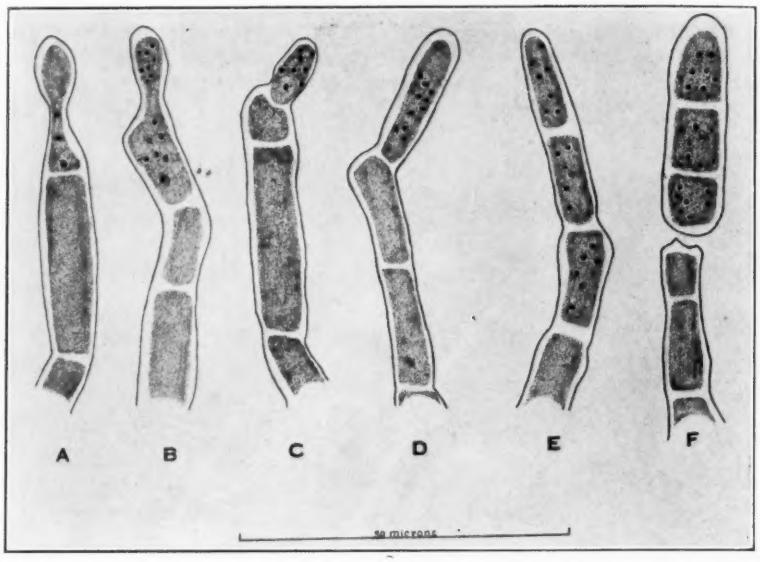

FIG. 2. The formation of conidia. Migration of several nuclei into the young spores shown at A, B, and C. Subsequent stages in the maturation of spores indicated at D, E, and F. Camera lucida drawings. Approx. × 1000.

seems reasonable, therefore, to suppose that the result may be the variation which is so characteristic of the organism.—T. W. GRAHAM, University Farm, St. Paul, Minn.

BOOK REVIEW

Goodey, T. *Plant Parasitic Nematodes.* xx + 306 pp. 136 figs. Methuen and Co., Ltd., London. 1933.

In this book is brought together for the first time the extensive and widely scattered information pertaining to the relationship between nematodes and plants. In the words of the author, the purpose of the book "has been to bring within the compass of a single volume the salient facts concerning all the known plant parasitic nematodes both as specific organisms and as producers of disease."

In chapter I are discussed the principal anatomical features of a typical nematode, methods for collecting and preparing for study specimens of plant-infesting species, methods commonly used for recording measurements and proportions, the present taxonomic status of the more important plant parasites, and morphological characters by which the genera Anguillulina, Heterodera, Aphelenchus and Aphelenchoides may be distinguished.

In the subsequent chapters, each of the more important parasites is discussed under the headings: historical, morphology, life-history, symptoms, pathology, hosts, geographical distribution and control, in so far as information on these phases is available. To this may be added a discussion of other facts peculiar to certain species. The question of biologic races as exhibited by *Anguillulina dipsaci* and *Heterodera schachtii* is discussed in the concluding chapter.

The author exhibits a thorough familiarity with the scattered and difficult literature on this subject: the material is well chosen and the subject matter well arranged. The press work is excellent with a commendable absence of typographical errors. The text is adequately illustrated with halftone and line cuts of a uniformly high grade. Each chapter is supplemented with a list of the more important literature references. The simple and readable style employed should make the book of value to the teacher, the general scientist, and the extension worker. The time consumed and labor expended in assembling information pertaining to plant parasitic nematodes have long been a distinct handicap to the specialists in this field. With a growing appreciation for the importance of diseases caused by these organisms, the need for such a text was becoming acute. It is fortunate that this book fulfills that need so well.—J. R. CHRISTIE.